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PROCEEDINGS OF THE FIFTH ANNUAL GENERAL MEETING OF BIOMINET

FOREWORD

BIOMINET presents in this volume the technical papers given by members of BIOMINET at the Fifth Annual General Meeting, held at the Crowchild Inn, Calgary, Alberta on November 2, 1988.

R.G.L. McCready
Editor

COMPTE RENDU DE LA CINQUIÈME RÉUNION GÉNÉRALE ANNUELLE DE BIOMINET

AVANT-PROPOS

Ce volume comprend les exposés techniques présentés par les membres de BIOMINET à la Cinquième réunion générale annuelle qui s'est tenue le 2 novembre 1988 au Crowchild Inn de Calgary (Alberta).

R.G.L. McCready
Éditeur

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SESSION I

BIOTECHNOLOGICAL APPLICATIONS TO MINE EFFLUENTS

SESSION I: PAPER 1

HEAVY METAL REMOVAL FROM GOLD MINING AND TAILING EFFLUENTS USING INDIGENOUS AQUATIC MACROPHYTES (PHASE 1)

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ABSTRACT

Gold mining has been conducted in Nova Scotia since the mid-1800s. Much of the province is underlain by rocks of the Meguma group of slates (Halifax series) and greywackes (Goldenville series). These formations often have gold and arsenic associated with them in the form of arsenopyrite. A Nova Scotia mining company plans to reclaim gold from seven abandoned tailing sites along the Black Brook near Oldham, Nova Scotia. The recovery of gold in this area will expose tailings to oxidizing conditions, resulting in serious ecological consequences to aquatic ecosystems downstream of these sites. A previous study of Black Brook showed that it was a stressed ecosystem, with several aquatic plant species exhibiting high concentrations of mercury and arsenic. Diversity of aquatic fauna was low, and several fish species exhibited histopathological abnormalities related to heavy metal toxicity and low pH.

The purpose of this study (Phase 1) was to characterize mineralogical and biological conditions of the site to establish an ecological understanding for using aquatic macrophytes to remove heavy metals from the proposed tailings excavations. Data were collected from 123 sampling stations distributed along 17 transects which crossed the tailings pond roughly perpendicular to Black Brook.

Very high values of mercury were found, ranging from 2.1 to 9.0 ppm in all samples. This is at least two orders of magnitude higher than the host rock. Arsenic values ranged from 25 ppm to 2.4 weight per cent (24000 ppm) and were consistent with being bound in the mineral arsenopyrite. The vegetation data were sorted into groups of similar species composition by using cluster analysis. This methodology provides an objective partitioning of the study area into distinctive plant communities, which can then be mapped relative to the transects. Data pertaining to the physical environment of the tailings (arsenic and mercury concentrations, per cent soil organic matter content, thickness of organic matter layer in the soil, depth to water table, and the vegetation data) were subjected to discriminant analysis.

In preparation for possible initiation of the Phase 2 study, which will investigate the capability of aquatic and semi aquatic macrophytes to remove arsenic and mercury from solution, twelve species of plants have been propagated in the laboratory.

SESSION I : EXPOSÉ 1

EXTRACTION DES MÉTAUX LOURDS PROVENANT DES EFFLUENTS DE RÉSIDUS MINIERS PAR LES MACROPHYTES AQUATIQUES INDIGÈNES (PHASE 1)

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RÉSUMÉ

L'exploitation de gisement aurifères a commencé vers le milieu du 19^{e} siècle en Nouvelle-Écosse. La majeure partie de la province est couverte par les ardoises du groupe Meguma (séries Halifax) et par les grauwackes des séries Goldenville. Ces formations renferment souvent de l'or auquel est associé de l'arsenic sous forme d'arsenopyrite. Une compagnie minière néo-écossaise se propose de récupérer l'or des résidus miniers abandonnés à sept emplacements le long du ruisseau Black, près d'Oldham en Nouvelle-Écosse. L'extraction de l'or dans cette région s'effectue en oxidant les résidus miniers, ce que entraîne de sérieuses conséquences écologiques pour l'environnement aquatique à l'aval des sites d'exploitation. Une étude précédente du ruisseau Black a demontré que l'écosystème a été sévèrement affecté; plusieurs plantes aquatiques exhibent de fortes concentrations de mercure et d'arsenic. La diversité de la faune aquatique est faible et plusieurs espèces de poisson montrent des signes histopathologiques d'intoxication par les métaux lourds et en raison du pH peu élevé de l'eau.

L'objectif de cette étude (Phase 1) était de caractériser les conditions biologiques et minéralogiques du site, et de déterminer le cadre environnemental préalable à l'utilisation de macrophytes aquatiques pour retirer les métaux lourds qui seront libérés par l'oxidation des résidus miniers. Les données ont été recueillies à 123 stations d'échantillonnage réparties en 17 transections traversant les étangs de résidus, perpendiculairement à l'axe du ruisseau Brook.

L'étude a permis de détecter de très fortes concentrations de mercure allant de 2,1 à 9,0 ppm dans tous les échantillons analysés. Les concentrations sont au moins 2 ordres de grandeur supérieurs à celles de la roche mère. Les niveaux d'arsenic sont de 25 à 24 000 ppm (2,4% en poids), ce qui s'explique par leur liaison à l'arsénopyrite. Les données sur la végétation ont été classées par groupes d'espèces semblables à l'aide d'une analyse par grappes. Cette méthode permet une répartition objective des diverses populations de plantes dans le territoire à l'étude, lesquelles peuvent être cartographiées en fonction des transections. Les données s'appliquant à l'environnement des résidus miniers (concentrations de mercure et d'arsenic, contenu de matière organique dans le sol, épaisseur de la couche de matière organique dans le sol, profondeur de la nappe phréatique et les données sur la végétation) ont fait l'objet d'une analyse de discriminant.

La Phase II de l'étude est une recherche sur la tendance des plantes semi-aquatiques macrophytes à retenir dans leurs tissus l'arsenic et le mercure en solution dans l'eau. Pour cette seconde phase, douze espèces de plantes ont été sélectionnées et cultivées en laboratoire.

HEAVY METAL REMOVAL FROM GOLD MINING AND TAILING EFFLUENTS USING INDIGENOUS AQUATIC MACROPHYTES (PHASE 1)

INTRODUCTION

Much of the province of Nova Scotia is underlain by rocks of the Meguma group of slates and greywackes which often produce gold. In the mid–1800s gold deposits were discovered, resulting in a series of intermittent gold rushes which continued until the 1940s. Part of the legacy of these mining operations is a large number of tailings deposits containing high concentrations of arsenic derived from the arsenopyrite associated with the gold deposits. Tailings deposits dating back to the end of the 19th century also contain high concentrations of mercury, derived from the mercury amalgamation technique used to separate the gold from the tailings. This technique was relatively inefficient compared to modern methods, and recently it has become potentially profitable to reprocess the tailings from these old mine sites to extract the residual gold.

One such tailings site is located at Oldham, Nova Scotia. The tailings are divided into seven separate deposits spread along the course of Black Brook, a small stream stressed by acid drainage within the watershed, and by arsenic and mercury derived from the tailings deposits. Black Brook eventually empties into the Shubenacadie River, which provides drinking water for several communities and supports significant commercial and sport fisheries. A Nova Scotia mining company plans to reclaim tailings from the largest of the tailings deposits. This would expose the remaining tailings to oxidizing conditions and would lead to increased losses of arsenic and mercury from the site, possibly resulting in serious consequences for aquatic ecosystems downstream.

A two–phase study was initiated, to characterize the mineralogical and biological conditions of the site (Phase 1) and to investigate the feasibility of using aquatic and/or semiaquatic macrophytes to remove arsenic and mercury from the waters draining the excavation site (Phase 2). Phase 1 has recently been completed and it is reported here. The Phase 1 study was designed to address the following objectives:

- to conduct a geological survey of a tailings site including a description of arsenic and mercury in tailings, with depth profiles;

- to describe the water quality of the study area including the quality of groundwater and receiving waters; and

- to describe the plant communities associated with the study area. This objective includes mapping of plant communities, determination of arsenic and mercury concentrations in plants, and measurement of the biomass of dominant terrestrial and aquatic plants.

In addition, we have attempted to ascertain what physical features of the tailings influence the species composition and productivity of the plant communities on the existing tailings deposits. This information is useful in selecting species to recolonize the remaining tailings area, following excavation of the more valuable tailings.

METHODS

SAMPLING PLAN

A field reconnaissance of the study site was conducted in early July 1988. At that time, biological and geological personnel involved in the project inspected the tailings area and devised a sampling program that integrated biological, geological and water chemistry sampling. In this way the interaction of the physical and biological systems on the site could be ascertained.

The sampling methodology chosen involved a series of 123 sampling sites distributed along 17 transects which crossed the tailings pond roughly perpendicular to Black Brook (Fig. 1). Biological data were collected at all sampling stations, and geological data were collected from a subset of 37 of the sampling sites. Six piezometers were installed in the tailings area to collect groundwater samples for chemical analysis and to permit monitoring of groundwater fluctuations during the field season. At each piezometer site, biological and geological data were collected. Surface water chemistry data were collected at five sampling sites upstream, within the tailings and downstream of the tailings.

TAILINGS COMPOSITION

At each geological sampling station a 70 cm hole was dug and samples of each distinctive stratum were collected. An average of four strata were found in each hole, for a total of 140 samples analyzed.

MINERALOGY

Mineral identification was done using a petrographic microscope of polished grain mounts in reflected light and examining loose grains under a binocular microscope. Grain mounts were prepared of bulk samples. Grain mounts were also examined of mineral separates made by Franz magnetic separator and by heavy liquids (Sp Gr = 2.7).

CHEMICAL ANALYSIS

Analyses for arsenic and mercury were performed by the Technical University of Nova Scotia Mineral Engineering Centre. Both elements were analyzed by atomic absorption analyses on uncrushed sample. Digestion was complete with respect to arsenic (aqua regia solution) and apparently complete for mercury (less powerful acid). A crushed sample was submitted to assure complete dilution, and no significant difference in results was seen. Sample handling was kept to a minimum, and crushing was not done. Visual inspection of mercury solutions was undertaken to verify complete digestion. The high values for mercury attained decrease the amount of concern for volatilization of the mercury in the sampling and preparation process. Most processed and least processed duplicate samples returned similar values. Blind duplicates were submitted to the analytical lab in addition to the laboratory's internal duplicates. Duplicate samples were submitted in each batch, and the same sample was submitted in several batches. Lab standards were run and reported, as were blind and lab blanks. Duplicate sample results, standards and blanks were within acceptable levels.

WATER CHEMISTRY

Groundwater samples were taken from six piezometers (Table 1) on three occasions during the summer. Surface water samples were taken from five fixed sampling sites upstream, downstream and within the tailings twice in the summer and once in the autumn. Surface water and groundwater samples were analyzed for major ions, metals and general chemical analysis. Groundwater samples were filtered before analysis, while surface water samples were not. Mercury samples were field preserved. Groundwater mercury samples were filtered before preservation. All analyses were performed at the Environmental Chemistry Laboratory, Victoria General Hospital, Halifax, Nova Scotia.

BIOLOGICAL SAMPLING

Locations of the 123 biological sampling sites are presented in Figure 1. Each sampling site consisted of a 50 cm^2 quadrat nested in a 2 m^2 quadrat. All ground vegetation and shrubs or trees under 50 cm in height were harvested in each 50 cm^2 quadrat, sorted by species, dried and weighed to the nearest milligram.

Shrub biomass was determined in the 2 m^2 quadrats. Direct harvesting of shrub biomass was not feasible because of the large volumes of shrub material present at many of the sampling stations. Instead, shrub biomass was estimated using biomass regression equations which estimate the biomass of a particular shrub from its diameter at 25 cm above-ground. The regression equations for conversion of diameter class measurements to biomass were calculated from specimens collected on site for *Alnus rugosa* (speckled alder), and *Spiraea latifolia* (hardhack), and from specimens collected off site for other studies for *Acer rubrum* (red maple), *Prunus pensylvanica* (pin-cherry), *Lonicera canadensis* (American fly-honeysuckle), and *Abies balsamea* (balsam fir) (3).

For reasons of data availability, and because of similar growth forms, equations derived for one species were sometimes used on others. *Acer rubrum* (red maple) was used for *Amelanchier* sp. (shadbush), *Prunus pensylvanica* (pin-cherry) was used for *Prunus virginiana* (choke-cherry), and *Lonicera canadensis* (American fly-honeysuckle) was used for *Lonicera villosa* (Mountain fly-honeysuckle). All equations are of the form:

$$\ln(\text{biomass}) = a + b\,[\ln(\text{diameter})] \qquad \text{Eq. 1}$$

The units are g/m^2 and cm, respectively.

The following table indicates the species for which the equation was derived, the number of measurements included, the adjusted r^2 (the estimated r^2 for the population, not the realized r^2 of the sample), the a and b values for the general equation (Eq. 1), and the species to which the equation was applied.

Source species	n	adj r^2	a	b	Used for
Acer rubrum	44	.9826	4.05458	2.49405	*Acer rubrum* *Amelanchier* spp.
Alnus rugosa	26	.9897	3.55096	2.88225	*Alnus rugosa*
Lonicera canadensis	28	.9532	4.41473	2.25260	*Lonicera villosa* *Rosa carolina*
Prunus pensylvanica	37	.9904	3.89179	2.56755	*Prunus virginiana* *Salix humilis*
Spiraea latifolia	27	.9614	4.32309	2.41333	*Spiraea latifolia*

Conversion of diameter measurements for *Picea glauca* (white spruce) was accomplished using equations from Freedman *et al.* (5).

At each sampling site the diameters of all shrubs within the 2 m^2 quadrat were recorded. These diameter measurements were then entered into the regression equations to calculate the weight of each shrub. Shrub weights were, in turn, summed to give the total above-ground shrub biomass for each sampling site.

Additional data collected at each sample site included the depth to the water table, depth of the organic matter layer, and the percent organic matter content of the upper 10 cm of the soil. To determine water table depth a metal pole was used to create a small well, approximately 50 cm deep and 2–3 cm wide. The water level in each well was allowed to equilibrate for a minimum of 30 minutes, at which time depth to the water table from the soil surface was measured with a meter stick. At most sampling sites, a distinctive brown humus layer was present on top of the grey tailings. Using a modified bulb planter, the thickness of this organic matter layer was measured from a 10–cm long soil core taken at each sampling station. The per cent organic matter content of each core was determined by loss on ignition.

Samples of vegetation found on the tailings and in Black Brook were analyzed for arsenic and mercury. The species tested included *Microspora quadrata, Equisetum fluviatile* (water horsetail), *Juncus articulatus* (rush), *Juncus pelocarpus, Dulichium arundinaceum, Leersia oryzoides* (rice cut-grass), and *Sparganium fluctuan* (bur-reed). A minimum of five individual plants of each species was collected from various locations in the study area. Where possible, roots and shoots were collected. The plants were carefully washed to remove attached soil particles. Roots were separated from shoots, and the samples were dried, homogenized and sent to the Victoria General Hospital Environmental Chemistry Laboratory in Halifax for arsenic and mercury analysis.

DATA ANALYSIS

All the statistical analyses were run using SPSS/PC+ Version 2.0. The 123 sites were clustered into communities based solely on biomass data using the procedure Cluster. The data were first transformed by taking the log natural of 1 plus the biomass, thus reducing the effect of extreme values and equating absent species to 0. Squared Euclidean distance and the centroid clustering algorithm were specified. Of the 123 sites, 120 were clustered into seven communities (leaving three outliers) based on examination of the cluster diagram.

The biomass data were then run through SPSS/PC+ discriminant analysis using these seven communities and principal component analysis. The results indicate that this cluster assignment was internally consistent.

The discriminant analysis was then used on the subset of 36 sites for which arsenic and mercury data were available. Three other physical factors (water table level, organic layer thickness and organic matter content) were also included. The arsenic and mercury values were logged (ln) to prevent the range and magnitudes of these variables from swamping the analysis (arsenic ranges from 25 000 – 24 000 ppm).

RESULTS AND DISCUSSION

MINERALOGY OF THE TAILINGS

The predominant mineral constituent of the tailings was vein quartz. Grains of vein quartz commonly have included sulphides – usually arsenopyrite but also pyrite and minor pyrrhotite. Chlorite was also a minor included mineral in the quartz. The next most common grains in the tailings were rock fragments. These fragments were micaceous (muscovite and chlorite) metasedimentary rocks that host the quartz veins at Oldham. The rock fragments contained sufficient magnetic oxides to make them easily separated by Franz magnetic separator. Only rarely did the rock fragments contain visible sulphides.

The next most common mineral constituents were a suite of iron and titanium oxides. They were magnetite, titanomagnetite, ilmenite and rutile. They were found as separate grains but most commonly as inclusions in all other grain types, most abundantly in the rock fragments. Sulphides were common as well and vary considerably in abundance from sample to sample. By far the dominant sulphide was arsenopyrite. Pyrite and pyrrhotite were subordinant and chalcopyrite was common as small inclusions in pyrite. Trace amounts of galena and sphalerite were also present.

Accessory minerals were very minor. Vein feldspar and vein carbonate (probably calcite, but Fe and Mn content are common in analyses of other gold district minerals in Nova Scotia) were identified. Secondary carbonate as hardpan has been seen in some of the sample sites near the level of the summertime water table. A few small gold grains have been seen but only as inclusions in arsenopyrite with quartz. The quality of polish of the gold grains was not sufficient to allow estimation of the mercury content. No liquid mercury was observed.

Most of the grains were unaltered although a few of the iron oxide grains had thin hematite (oxidized Fe oxide; Fe_2O_3) rims. Sulphides were typically not altered.

CHEMICAL ANALYSIS OF THE TAILINGS

Arsenic and mercury analyses from the main tailings accumulation at Oldham indicated significant concentrations of both metals: mean concentration of mercury 5.45 parts per million (ppm) (one standard deviation = 3.66 ppm where n = 131) and mean concentration of arsenic 1900 ppm (one standard deviation = 3200 ppm where n = 131). Mercury values ranged from 1.1 to 19.9 ppm with all values elevated at least two orders of magnitude from expected background in the surrounding rock that was mined for gold ore. Two bedrock samples analyzed in this study were 0.13 and 0.10 ppm mercury and 450 and 275 ppm arsenic. Arsenic concentration in the tailings ranged from 25 ppm to 2.4 weight per cent (24 000 ppm). These extreme values should be expected because the tailings are

the waste from a vein quartz – arsenopyrite (FeAsS) – gold concentrate in which everything but gold was waste. The mined material was hand separated, both underground to reduce haulage and at least once on the surface to decrease millage. Pieces of quartz with visible gold, arsenopyrite (associated spatially with gold throughout Nova Scotia) and vein quartz would have constituted the mill feed.

Four grain-size fractions were analyzed from a sample with low mercury and arsenic concentrations. Both arsenic and mercury increased with decreasing grain size in the one experiment conducted. Similar variation of increasing mercury with decreasing grain size was seen in many of the individual sample profiles, but this relationship was not evident in the arsenic analyses.

Arsenic and mercury concentrations in dry sandy tailings were enriched in areas of uniform sandy tailings of higher gold value. This represented a primary distribution of these components resulting from mining and disposal geometry. Arsenic distribution and concentration were consistent with being bound mostly in the stable sulphide mineral arsenopyrite. Sand-sized arsenopyrite grains and arsenopyrite inclusions in sand-sized quartz grains were abundant in the tailings. The source of the arsenopyrite was the local rock that was mined and milled with the arsenopyrite as a waste (tailings) product. The mercury was introduced into the tailings by inefficient recovery of mercury from the amalgamation process. Mercury is present in only negligible amounts in the local bedrock. Neither mercury minerals nor liquid mercury were observed. Distribution and concentration of mercury were consistent with the mercury residing in the tailings as fine grains or adhered to fine grains in the clay silicate fraction and in the fine-grained organic fraction. This results in mercury enrichment in the soil horizon, buried soil horizons, and basal clay layers or clay substrate. Mercury lost during the milling process was most likely liquid mercury and particles of mercury amalgamated with gold within the tailings. Inefficient retorting could have added more mercury by air but it is difficult to assess this source.

Redistribution of arsenic within the tailings appeared minor, although erosion of solid tailings particles downstream away from the tailings may increase the mobility of arsenic. Redistribution of the mercury within the solid tailings was evident. In distal tailings, with lower bulk composition of mercury and arsenic and a finer grain size, mercury was enriched in the upper samples of a profile. In proximal tailings, mercury was often depleted in the upper samples. Mechanical redistribution by wind would account for this distribution. The role of vegetation density and type appeared important as well, and a more complete discussion is found below.

Sample depth had a variable effect on mercury and arsenic concentrations. Sample sites in the southern alder-covered part of the tailings often had multiple profiles separated by a soil/organic horizon. This probably represents two different times of tailings addition. The northern half of the tailings accumulation had simpler profiles. A typical profile had an organic-rich soil layer at the top 5 to 15 cm. Enrichment of mercury was common in this upper zone and is discussed below. A grain size profile would include a fine-grained organic top, often depleted in arsenic and enriched in mercury relative to lower samples, a grey (quartz) and black (arsenopyrite, oxides, and rock fragments) sand containing most of the arsenic, and underlain by a grey clay with or without organic material (the pre-tailings substrate or soil) with high mercury and little arsenic. The distribution of arsenic and mercury within this profile must thus consider grain size.

Lateral distribution of tailings should also be considered in terms of grain size variation. Relatively coarse-grained (medium-grained sand) proximal tailings constitute the ore (in terms of gold) to the southwest corner and west margin of the accumulation. Finer grained and thinner tailings are in the east and north of the accumulation.

The average grain size of each sample was estimated. Higher mercury values were, in general, found in finer grained samples both throughout the tailings accumulation and within any profile. Medium-grained sand samples had mean values of 4.35 ppm mercury (1 standard deviation 3.05 ppm for 51 samples), while silt-sized to clay-sized samples had mean values of 6.22 ppm (1 standard deviation 3.17 ppm for 19 samples). Soil samples with organic material volumetrically important also had higher mercury values: a mean of 5.80 ppm (1 standard deviation of 2.44 ppm for 10 samples). Arsenic concentration was higher in the sand-sized fractions (mean 2580 ppm) as opposed to finer grained samples (fine sands, silts, and soils average 1140 ppm). Arsenic values for clay-sized samples were low, with mean values of 620 ppm.

The mercury distribution in surface samples is clearly influenced by biotic factors. The more plant cover over tailings, the more likely that the mercury levels are elevated. This relationship can be seen by comparing the mercury values in various plant communities or by considering vertical tailings profiles of sample stations. All results in excess of 5 ppm mercury are from samples taken in wet communities with ample plant cover (Fig. 2). Mercury enrichment in surface samples is apparently unrelated to the rest of the vertical tailings profile.

These observations can most easily be explained by mechanical trapping of wind-blown mercury-rich fine sediment by plants. Part of the tailings accumulation has little or no plant cover and provides a supply of mercury bound in fine wind-blown particles. Fixing of the entire tailings accumulation would obviously decrease the supply and mobility to wind of fine-grained sediment. Plant cover could also be expected to decrease the supply of the same sediment to stream erosion. This same relationship is not obviously seen in the case of arsenic. Either it is less concentrated in the fine fraction of the tailings or fine-grained arsenic minerals are more mobile with respect to groundwater than mercury and are removed by solution.

GROUNDWATER

Table 1 presents groundwater chemistry data for samples from six piezometers located on the study site (Fig. 1). The groundwater typically had high concentrations of calcium, which contributed to the high hardness and pH values noted in all samples. The high pH would also increase the mobility of cations, such as arsenic, iron and manganese, all of which exceeded recommended concentrations at all sites and on all sampling dates. Concentrations of copper and zinc were slightly in excess of recommended values for piezometers 1, 2 and 4. Mercury, although highly concentrated in the tailings, was generally not detectable in the tailings groundwater, suggesting that it was tightly bound to soil particles and subject to very slow weathering. Mercury in excess of recommended values was found on only one date. High concentrations of other metals were not detected.

Temporal fluctuations in cation and anion concentrations in the groundwater were generally low, with the exception of arsenic and iron. Concentrations of both cations were low on June 10 and high on June 24 and August 12. Water table depth data from the piezometers indicated that the former dates corresponded to low water periods. A low water table would permit weathering of tailings which are normally waterlogged for much of the year, allowing a flush of arsenic and iron into the tailings groundwater.

Water table depth was measured for all 123 sampling sites on September 21, 1988. Depth to water table ranged from 70 cm below surface to 38 cm above surface, with an average depth of 12 cm below surface. Fluctuations in water table depth were monitored at the six piezometers. Temporal variations in water table depth between June 3 and September 21, 1988 ranged from 54 cm at

piezometer 2 to 20 cm at piezometer 5, with an average for all sites of 35 cm (Table 2). Fluctuations in water table depth were often rapid as a result of the high porosity of tailings. Fluctuations of up to 8 cm per day were noted, although the average rate of fluctuation for most of the piezometers was about 3.5 cm per day.

SURFACE WATER CHEMISTRY

Table 3 presents surface water chemistry data for five sampling sites on three occasions. Locations of sampling stations are presented in Figure 1. Data for Site 1 upstream of the tailings on Black Brook indicated an oligotrophic stream subjected to acid stress. The high acidity resulted from acid drainage from exposed slates at the headwaters of Black Brook (Halifax International Airport). Acidic conditions permit the mobilization of metals such as aluminum, which were highly elevated at Site 1. Copper and chromium were also elevated at this site. As water from Black Brook passes through the tailings (Sites 2 and 3), pH increases, probably as a result of inputs of calcium from tailings groundwater. Data for June 6 indicate that the tailings can significantly alter the pH of Black Brook under certain circumstances. In most instances, however, reductions in pH were much less drastic. Concentrations of aluminum tended to decline somewhat as Black Brook passed through the tailings. Aluminum concentrations remained above recommended values at all Black Brook sampling sites in spite of these reductions.

Arsenic concentrations were below detectable limits at Site 1, but they increased to beyond acceptable concentrations at Sites 2 and 3. Inputs of tailings groundwater enriched with arsenic were probably responsible for the increase in arsenic concentrations in Black Brook. Similar inputs of manganese were also noted. Contributions of arsenic from the tailings were highest in the summer months, particularly when stream water flow rates were low and inputs of tailings groundwater formed a relatively high proportion of the water entering the brook.

A small proportion of the surface water passing through the tailings came from a small stream entering the pond in the eastern portion of the study area. Water chemistry from this stream (Site 4) exhibited no water quality parameters other than colour exceeding recommended values. This stream subsequently passed through a small tailings deposit and entered the pond situated on tailings in the study site. Water in the pond was enriched in arsenic and iron by passage through the upstream tailings and probably through ion exchange with the tailings underlying the pond. Concentrations of these cations exceeded recommended values on all sampling dates with the highest concentrations occurring in late summer when stream flow was low.

These data indicate that the tailings deposits on Black Brook have both positive and negative impacts on stream water quality. The tailings reduce stream water acidity; however, they increase concentrations of arsenic and iron. The net effect is negative, since pH remains suboptimal after passage of stream water through the tailings, and concentrations of arsenic and iron are increased to levels harmful to stream biota.

SOIL ORGANIC MATTER

Soil organic matter content was generally low, averaging 5.7% for all of the sites. The lowest values (0.1%) were found in areas bare of vegetation, while the highest values were found in marshy alder thickets (61.5%) and along the banks of Black Brook (36.1%). In both of the latter locations, inputs of organic matter were high and decomposition rates were low. At most sampling sites, soil profiles had an organic mat at the surface in which most of the root systems of the resident plants were

located. The thickness of this mat varied widely over the study area, usually displaying a pattern similar to that of organic matter content.

THE BIOTIC ENVIRONMENT

The tailings deposits at Oldham are well vegetated in comparison to many other tailings areas in Nova Scotia. In most areas vegetation coverage exceeded 100%. Species richness was high in most of the tailings plant communities ranging between 12 and 15 species/m², which is somewhat higher than the species richness of abandoned pasture and shrub thickets on sandy till in Nova Scotia. Table 4 presents a list of 77 species of plants noted in the study area. Many of the species dominant in the various plant communities described below are commonly found on abandoned mine tailings and are probably metal tolerant genotypes derived from local populations. The most widely distributed species on the tailings – *Equisetum fluviatile* (water horsetail), *E. arvense* (field horsetail), *E. variegatum* (horsetail), *Juncus effusus* (soft rush), *Agrostis palustris* (creeping bent–grass) and *Centaurea nigra* (knapweed) are known to be tolerant of high arsenic concentrations or have been shown to be tolerant of other heavy metals (2,4,7). Others, such as *Juncus articulatus* (rush), *Viola cucullata* (blue violet) and *V. pallens* (small white violet), belong to genera in which metal tolerant species have been found. None of the species listed in Table 4 are rare in Nova Scotia.

Table 5 presents concentrations of arsenic and mercury in the roots and shoots of a number of species found on the tailings at Oldham. Unfortunately, analysis of arsenic and mercury concentrations in the tissues of these species was conducted before the various plant communities on the tailings had been identified. Ideally, the dominant species of each community would have been analyzed to see how they vary in their ability to take up arsenic and mercury. All species analyzed accumulated high concentrations of both arsenic and mercury. Arsenic concentrations in the above–ground portions of the terrestrial and semiaquatic species ranged from 321 to 700 ppm, which is similar to ranges noted in other studies (2,4). Concentrations in the roots ranged from 4.4 to 17.0 times higher than concentrations in the shoots. This trend has been noted in other studies (1,8). Higher concentrations in the roots may be the result of adsorption of arsenic to the epidermis of the root which is constantly exposed to water relatively rich in arsenic. Concentrations of arsenic in the tissues of the filamentous green algae *Microspora quadrata* and the bur–reed *Sparganium fluctuans* were similar to those of the roots of the terrestrial plants. These plants may also adsorb large amounts of metals on their surfaces.

Concentrations of mercury in the roots and shoots of the various species exhibited trends similar to those found for arsenic. Concentrations were highest in the roots, and aquatic plants accumulated concentrations similar to those in the roots of terrestrial plants. These data suggest that most of the species found on the tailings are probably genotypes tolerant of high arsenic and mercury concentrations.

Table 6 presents a list of 27 species common in at least one of seven plant communities found on the tailings area. The criterion for inclusion in the list was a frequency of occurrence of no less than 30% in at least one of the plant communities. This eliminated species whose presence in a community may be the result of chance rather than that species' ability to survive under the environmental conditions prevalent in the community. The plant communities presented in Table 6 were derived from cluster analysis of the species by biomass matrix of all quadrats sampled during the study. Figure 3 presents a schematic of the cluster diagram. The first split in the diagram separated three sample sites containing unique species assemblages from the rest of the quadrats. These sample sites were classed as outliers and were eliminated from further analysis. The next split separated shrub dominated communities (Clusters 5 and 7) from communities dominated by herbaceous species (Clusters 1–4 and 6).

Subsequent splits within the shrub communities produced a well-defined cluster (Cluster 5) and a poorly defined set of sample sites closely related to Cluster 5, which for ease of analysis were lumped into a separate group (Cluster 7). The herbaceous communities were divided into four well-defined clusters (Clusters 1–4) and a poorly defined group of sample sites, each of which was distinctive from each other but closely related to the herbaceous sample sites. These sites were grouped together to form Cluster 6.

The data were then analyzed by discriminant analysis using the seven clusters as the grouping variable. Results of this analysis confirmed the findings of the cluster analysis and the decision to lump the poorly defined groups of sample sites into Clusters 6 and 7. Only one of 120 sample sites was misclassified according to the discriminant analysis. Figure 4 presents a plot of the first two canonical discriminant functions. These functions accounted for 73% and 11% of the variance. Function 1 separated herbaceous communities from shrub communities. Function 2 separated wet communities from dry ones.

Cluster 1 (Table 6, Fig. 1) represents a shallow pond plant community dominated by *Chara globularis*, *Equisetum fluviatile* (water horsetail) and *Utricularia intermedia* (bladderwort). Species richness was very low in this community (2.6 species/m^2) as was total above-ground biomass (122 g/m^2). The presence of permanent water appears to be the main determinant of the species composition of this community, since *Chara globularis* and *Utricularia intermedia* (bladderwort) are found only in permanent standing water.

Cluster 2 (Table 6, Fig. 1) was composed of quadrats dominated by *Juncus articulatus* (rush) and *Equisetum fluviatile* (water horsetail). These species accounted for 73% of total above-ground biomass for this community. Other minor species frequently found in this community type were *Agrostis palustris* (creeping bent-grass), *Hypericum virginicum* (St. John's wort), *Lycopus uniflorus* (bugle weed) and *Lysimachia terrestris* (loosestrife). *Eupatorium perfoliatum* (boneset) contributed considerable biomass (11%) but occurred relatively infrequently (29%). Most of the study sites in this community had water tables close to the surface (Table 7) reducing the rate of decomposition of organic material and permitting the build up of a relatively thick organic layer on the soil surface. The percentage of organic matter in the soil, however, was rather low, probably because the organic layer was composed of only partially rotted material that would be sieved out of the sample before loss on ignition. Arsenic concentrations in the soil tended to be moderate, while mercury was low.

In Cluster 3 (Table 6, Fig. 1) the dominant species were *Campylium stellata* and *Equisetum fluviatile* (water horsetail), which made up 81% of total above-ground biomass for this community. *Agrostis palustris* (creeping bent-grass), *Aster novi-belgii* (New York aster), *Equisetum variegatum* (horsetail), *Eupatorium perfoliatum* (boneset) and *Lycopus uniflorus* (bugle weed) were minor species frequently found in this community. The physical environment for plants in this community was characterized by very low soil organic matter content combined with moderate values for water table depth, arsenic and mercury concentrations (Table 7).

The species composition of Cluster 4 (Table 6, Fig. 1) was dominated by *Equisetum fluviatile* (water horsetail), *Agrostis palustris* (creeping bent-grass), *Equisetum arvense* (field horsetail) and *Eupatorium perfoliatum* (boneset). Together these species accounted for 76% of the total above-ground biomass. Minor species typical of this community included *Aster novi-belgii* (New York aster), *Juncus articulatus* (rush), *Lysimachia terrestris* (loosestrife) and *Viola pallens* (small white aster). Mean total above-ground biomass for this community was quite low, probably as a result of seasonal water stress, since the water table was deep and there was little organic matter in the soil to improve

its water holding capacity (Table 7). Arsenic and mercury concentrations were low, however, biomass production did not appear to be affected by these factors.

Cluster 6 (Table 6, Fig. 1) was the last of the herbaceous communities. *Equisetum fluviatile* (water horsetail), *Eupatorium perfoliatum* (boneset), *Equisetum arvense* (field horsetail), *Campylium stellata* and *Agrostis palustris* (creeping bent-grass) were the dominant species, comprising 51% of the total above-ground biomass. The relatively low proportion of the total above-ground biomass accounted for by these species may be attributable to the low uniformity in species composition for this community. As mentioned earlier, Cluster 6 is an aggregation of quadrats whose species compositions were generally not closely related to each other. Minor species frequently encountered in this community included *Aster novi-belgii* (New York aster), *Lysimachia terrestris* (loosestrife), *Viola cucullata* (blue violet) and *V. pallens* (small white violet). *Spiraea latifolia* (hardhack) contributed 14% of the total above-ground biomass although it was found in only 11% of the quadrats. *S. latifolia* is a shrub and is, therefore, capable of accumulating appreciable quantities of woody biomass over time, while herbaceous species are limited in the amount of biomass they can accumulate. Arsenic and mercury concentrations were extremely high in this community and the water table was very low; however, mean total above-ground biomass was higher than in any of the other communities dominated by herbaceous vegetation. This high productivity may be related to the large amounts of organic matter in the soil which help to retain water and provide a source of plant macronutrients. Organic matter can also absorb metals, lowering the concentration available for plant uptake (1).

Cluster 7 (Table 6, Fig. 1) represents a short shrub community dominated by *Alnus rugosa* (speckled alder) and *Spiraea latifolia* (hardhack), which together accounted for 75% of the total above-ground biomass for this community. Minor species that occurred regularly in this community included *Aster umbellatus* (tall white aster), *Equisetum fluviatile* (water horsetail), *Hypericum virginicum* (St. John's wort) and *Viola pallens* (small white violet). This community was characterized by a high water table and very high organic matter content (Table 7). The high water table would enhance colonization of this area by plants and would aid in the accumulation of organic matter. Nitrogen fixation by *Alnus rugosa* would improve site fertility, permitting the accumulation of a large standing crop of biomass. *Alnus* stems were relatively small in this community and appeared to be stressed. Shrub size and vigour were lowest in the wettest areas, suggesting that increased root respiration associated with water-logging was largely responsible for the stress. Arsenic concentrations were high in this community; nevertheless, a high standing crop of biomass was maintained in relation to other communities with considerably lower concentrations of arsenic. Crowell (3) found that deciduous shrub thickets of similar total above-ground biomass in Nova Scotia were composed of 80% woody biomass and 20% foliage biomass. Elimination of 80% of shrub biomass from the short A. rugosa – *Spiraea latifolia* community left approximately 540 g/m^2 of foliage biomass, which was higher than the total above-ground biomass of the herbaceous communities on the tailings. This suggests that the high average arsenic concentrations in the soil of this community had relatively little effect on site productivity. Arsenic was probably unavailable to the plants, because it was either bonded in relatively inert sulphide minerals or adsorbed to organic matter.

The sample sites from Cluster 5 (Table 6, Fig. 1) were occupied by a tall shrub community dominated by *Alnus rugosa* (speckled alder) and *Spiraea latifolia* (hardhack). These species contributed 97% of the total above-ground biomass of the community. *A. rugosa* stems were much larger in this community than in the previous community, and the distribution of *A. rugosa* was much less patchy; consequently, the contribution of this species to the total above-ground biomass was considerably larger in Cluster 5 than in Cluster 7. The water table at this site was lower than that in the short shrub community, so shrubs did not suffer from water-logging (Table 7). The amount of organic matter in the soil was high but lower than that in the short shrub community, consisting of well rotted humus rather

than coarse peat. This suggests that nutrient cycling was more efficient in this community, making more of the nutrients bound in the organic matter available for plant growth. As in the short shrub community, arsenic concentrations were high (averaging 1240 ppm); however, no reductions in plant productivity were apparent. Growth rates of the dominant *R. rugosa* (as determined by observation of annual rings) were good. These results suggest that most of the arsenic in the soil was unavailable for plant uptake.

The relative importance of physical environmental factors (depth to water table, organic matter depth, organic matter content and arsenic and mercury concentrations) in determining the vegetation composition of the Oldham tailings was determined by discriminant analysis using these variables as the discriminating variables. The first four discriminant functions explained 74%, 20%, 4% and 2% of the variance, respectively. The first discriminant function was most highly correlated with depth to water table, the second function was most highly correlated with thickness of the organic matter layer, the third function was highly correlated with arsenic concentration, and the fourth function was highly correlated with both mercury concentration and organic matter content. In Figure 5, the first discriminant function separated plant communities characteristic of wet areas (Clusters 1 and 2) from mesic (Clusters 3, 6 and 7) and dry areas (Clusters 4 and 5). The second discriminant function separated mesic study sites with thick organic layers (Clusters 6 and 7) from those with thin organic layers (Cluster 3) and dry sites with thick organic layers (Cluster 5) from dry sites with thin organic layers (Cluster 4).

The incidence of misclassification of study sites based on the discriminating variables was low (5 out of 36), indicating that physical features of the tailings site (particularly depth to water table and the thickness of the organic matter layer) could be used to accurately predict the type of plant community occupying the site. This provides indirect evidence that these physical features determine the nature of the vegetation on the site. It is important to realize, however, that the vegetation also influences the nature of the physical environment.

Although high concentrations of arsenic and mercury were present in the tailings, variations in their concentrations had little effect on plant community distributions. This was probably attributable to discrepancies between the total amount of arsenic and mercury in the tailings and the amount of each available for plant uptake. Most of the arsenic and mercury in the tailings is not easily weathered and is, therefore, unavailable to plants. The rate at which each is weathered may vary from place to place in the tailings, and movement of groundwater through the tailings may transport available arsenic and mercury some distance before it is taken up by plants. Organic matter in the soil forms stable complexes with metal ions, making them unavailable to plants (6). The high productivity of the vegetation in Clusters 5, 6 and 7 in spite of very high arsenic and mercury concentrations in the soil is probably attributable to complexing of arsenic and mercury with organic matter. The net effect of these factors would be to destroy any correlation between total arsenic and mercury concentrations in the tailings and the concentrations of arsenic and mercury that are available for plant uptake at any particular site.

The chemical nature of the tailings and the tailings groundwater can ameliorate the toxic effects of these metals on the vegetation, weakening the relationship between metal concentration and species composition. High concentrations of calcium have been shown to inhibit the uptake of a number of heavy metals by effecting cell permeability (1). High concentrations of non-toxic cations may also reduce metal toxicity by reducing the net concentrations of toxic ions in solution, or they may interfere with uptake mechanisms by competing for entry sites. The relatively high concentrations of cal-

cium in the Oldham tailings groundwater may reduce the toxic effects of arsenic and mercury in the vegetation.

Table 8 indicates habitat preferences of the common species on the Oldham tailings by listing the physical characteristics of the plant community in which the various species reach their maximum biomass and frequency. Soil arsenic and mercury concentrations proved to be of little value in predicting species composition and have, therefore, been eliminated as descriptors of species habitat preferences. A list of habitat preferences of the common species on the Oldham tailings is presented in Table 8. Knowledge of the habitat preferences of metal tolerant species such as are found on the Oldham tailings is useful in selecting plant material for revegetation of disturbed or newly formed tailings deposits. Under such conditions, a species capable of growth in a substrate low in organic matter would be useful. On dry tailings (average summer water table depth = 20+ cm), *Agrostis palustris* (creeping bent–grass), *Aster novi–belgii* (New York aster), *Centaurea nigra* (knapweed) and *Equisetum arvense* (field horsetail) could be expected to perform well. *Centaurea nigra*, *Agrostis palustris* and *Equisetum arvense* are known to be arsenic accumulators. At Oldham, *Agrostis palustris* and *Equisetum arvense* were the first species to colonize bare areas. In mesic areas (average summer water table depth = 10–20 cm), *Campylium stellata*, *Chrysanthemum leucanthemum* (ox–eye daisy), *Equisetum fluviatile* (water horsetail), *Equisetum variegatum* (horsetail), *Lycopus uniflorus* (bugle weed) and *Prunella vulgaris* (heal–all) should grow well. *Equisetum fluviatile*, in particular, is well adapted to the tailings. It has a very high tolerance for arsenic and can tolerate both water–logged and very dry conditions. *Equisetum fluviatile* individuals at Oldham had extensive rhizome systems that extended as deep as 50 cm into the tailings. All other species had their roots restricted to the upper 10 cm of the tailings and were, therefore, more subject to water stress. In wet areas (average summer water table <10 cm), *Equisetum fluviatile*, *Chara globularis* and *Utricularia* intermedia (bladderwort) could be grown. Growth of Chara and Utricularia would be restricted to permanently flooded areas. *Chara* is generally restricted to waters of relatively high pH so the acidity of the surface waters would have to be considered before choosing this species; however, it has been shown to be an excellent metal accumulator. *Utricularia intermedia* is tolerant of low pH and obtains much of its nutritional requirements through insectivory; however, growth rates are usually quite low.

Species typical of areas with moderate to high organic matter content would probably require the addition of fertilizer or organic matter in the form of manure, sewage sludge, peat or forest floor duff to the tailings to permit maximum growth. Some species such as *Juncus articulatus* (rush), *Eupatorium perfoliatum* (boneset) and *Lysimachia terrestris* (loosestrife), however, can survive on tailings with low organic matter, and could be sown with species tolerant of low soil organic matter, so that, as organic matter is built up, these more productive species would be available to colonize it. Nitrogen fixers such as *Alnus rugosa* (speckled alder) would help to alleviate nitrogen deficiencies on tailings sites, but would require a large initial application of organic matter to ameliorate seasonal water stress and provide other essential nutrients.

CONCLUSIONS

The most significant finding of the tailings analyses is the high and consistent amount of mercury. Mercury distribution is controlled by:

a) tailings grain size (the finest grain portions of a tailings profile tend to be more mercury rich),

b) grade of tailings (tailings associated with ore–grade areas are more mercury rich).

c) amount of fine-grained organic matter (the more organic rich samples, especially in generally fine-grained tailings tend to be more mercury rich).

Arsenic concentration and distribution are controlled by arsenopyrite distribution. Redistribution into the fine organic or clay fraction is not evident.

The groundwater chemistry of the tailings is characterized by concentrations of arsenic, iron and manganese in excess of recommended values, but relatively little free mercury. The tailings contribute enough arsenic to Black Brook to increase downstream concentrations beyond safe limits; however, the buffering action of the tailings helps to alleviate the acid drainage problem experienced by this brook. The vegetation of the site appears to be composed largely of genotypes of relatively common species capable of tolerating high concentrations of both arsenic and mercury. The aquatic species tested accumulated very high concentrations of both metals, which may reflect both active metal uptake and passive adsorption of arsenic and mercury onto their surfaces.

A total of seven plant communities were noted in the tailings deposits. The most important physical features of the tailings environment determining the distribution of plant communities on the tailings were depth to water table and the thickness of the surface organic layer. Arsenic and mercury concentrations appeared to have little influence on the distribution of tailings plant communities, although they were often present in very high concentrations. The apparent lack of influence of these metals on plant community development on the tailings may be related to differences in the availability of these metals to plants in different locations (as a result of differences in weathering rates and adsorption of these metals to organic matter) and to the fact that most of the species present on the tailings were probably tolerant of arsenic and mercury contamination.

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TABLES

Table 1 – Water quality data for tailings groundwater at Oldham*

	P1			P2		
Date	10/6/88	24/6/88	12/8/88	10/6/88	24/6/88	12/8/88
Calcium	36.0	56.0	55.0	40.0	47.0	54.0
Magnesium	3.6	5.8	4.9	2.9	2.5	2.4
Hardness ($CaCo_3$)	104.8	163.7	157.3	111.7	127.5	144.9
Alkalinity ($CaCo_3$)	57.0	82.0	130.0	120.0	150.0	143.0
Sulphate	58.0	89.0	30.0	3.8	<1.0	12.0
Chloride	3.6	3.6	2.8	3.8	3.4	2.3
Ortho–Phosphorus (P)	0.22	1.5	3.2	0.16	0.27	0.19
Nitrate & Nitrite (N)	0.10	<0.05	<0.05	0.05	<0.05	<0.05
Ammonia (W)	0.30	0.32	0.42	<0.05	<0.05	0.06
Arsenic	0.080*	13.0*	18.1*	0.200*	0.790*	0.860*
Iron	0.25	19.0*	21.0*	0.04	2.3*	3.7*
Manganese	1.9*	2.3*	2.3*	1.0*	2.2*	2.0*
Copper	<0.01	0.01*	<0.01	0.02*	<0.01	–
Zinc	0.06*	0.01	<0.01	0.07*	0.02	0.01
Colour (T.C.V)	12.0	–	6.0	13.0	–	53.0*
Conductivity (micromho/cm)	242.0	345.0	310.0	232.0	274.0	292.0
pH (unit)	6.60	6.40*	6.70	7.30	7.50	7.20
Lead–ICP & HGA	<0.05	<0.002	<0.002	<0.05	<0.002	<0.002
Aluminum	0.05	0.05	<0.05	<0.05	0.06	<0.05
Boron	<0.02	<0.02	<0.02	<0.02	<0.02	<0.02
Barium	0.040	0.021	0.019	0.027	0.007	0.013
Beryllium	<0.005	<0.005	<0.005	<0.005	<0.005	<0.005
Chromium	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Cobalt	0.02	<0.01	–	<0.01	<0.01	<0.01
Nickel	0.02	<0.02	<0.02	0.03	0.02	0.02
Antimony	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05
Selenium	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
Tin	<0.03	<0.03	<0.03	<0.03	<0.03	<0.03
Vanadium	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Cadmium	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Mercury (ppm)	–	<0.05	<0.05	–	<0.05	<0.05
Sodium	–	2.5	–	–	2.5	–
Potassium	–	0.80	–	–	1.4	–
Total organic carbon	–	7.5	–	–	3.8	–

*Values exceeding recommended concentrations are marked with an asterisk. All values are in mg/L unless otherwise stated. Locations of piezometers (P1–P6) are presented in Figure 1

Table 1 (Cont'd)

	P3			P4		
Date	10/6/88	24/6/88	12/8/88	10/6/88	24/6/88	12/8/88
Calcium	131.0	112.0	104.0	143.0	142.0	153.0
Magnesium	2.3	2.1	1.9	2.5	2.5	2.4
Hardness ($CaCo_3$)	336.5	288.2	267.3	367.2	364.8	392.0
Alkalinity ($CaCo_3$)	370.0	300.0	273.0	390.0	370.0	370.0
Sulphate	<1.0	<1.0	<2.0	16.0	12.0	<2.0
Chloride	3.4	3.3	2.2	2.7	3.0	3.1
Ortho-Phosphorus (P)	0.32	<0.01	0.06	0.26	0.82	<2.0
Nitrate & Nitrite (N)	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05
Ammonia (W)	<0.05	<0.05	<0.05	<0.05	<0.05	0.10
Arsenic	12.2*	11.8*	32.0*	0.190*	5.7*	7.0*
Iron	41.0	75.0*	69.0*	0.10	26.0*	30.0*
Manganese	6.7*	3.8*	3.5*	3.0*	3.8*	3.8*
Copper	<0.01	<0.01	<0.01	0.03*	<0.01	<0.01
Zinc	0.01	0.02	0.01	0.01	<0.01	0.01
Colour (T.C.V)	480.0*	–	280.0*	<10.0	–	75.0*
Conductivity (micromho/cm)	732.00	626.0	553.0	767.0	745.0	832.0
pH (units)	7.00	6.90	6.70	7.30	7.00	6.90
Lead-ICP & HGA	<0.05	<0.002	<0.002	<0.05	<0.002	<0.002
Aluminum	<0.05	0.05	<0.05	<0.05	<0.05	,<0.05
Boron	<0.02	<0.02	<0.02	<0.02	<0.02	<0.02
Barium	0.094	0.113	0.127	0.069	0.103	0.130
Beryllium	<0.005	0.005	<0.005	<0.005	<0.005	,<0.005
Chromium	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Cobalt	<0.01	<0.01	–	<0.01	<0.01	–
Nickel	0.03	0.02	0.02	<0.02	<0.02	<0.02
Antimony	<0.05	<0.05	<0.05	<0.05	<0.05	,<0.05
Selenium	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
Tin	0.08	<0.03	0.07	0.09	<0.03	0.11
Vanadium	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Cadmium	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Mercury (ppm)	–	<0.05	0.13*	–	<0.05	<0.05
Sodium	–	3.5	–	–	2.5	–
Potassium	–	7.3	–	–	5.5	–
Total organic carbon	–	9.0	–	–	5.0	–

*Values exceeding recommended concentrations are marked with an asterisk. All values are in mg/L unless otherwise stated. Locations of piezometers (P1-P6) are presented in Figure 1.

Table 1 (Cont'd)

	P5			P6		
Date	10/6/88	24/6/88	12/8/88	10/6/88	24/6/88	12/8/88
Calcium	105.0	161.0	94.0	76.0	96.0	87.0
Magnesium	3.3	5.2	3.1	2.6	2.4	2.4
Hardness ($CaCo_3$)	275.6	423.6	247.6	200.4	249.7	227.3
Alkalinity ($CaCo_3$)	300.0	470.0	303.0	200.0	230.0	217.0
Sulphate	6.1	<1.0	<2.0	9.8	3.90	23.0
Chloride	3.2	2.1	5.0	2.7	4.1	2.9
Ortho-Phosphorus (P)	0.83	0.41	0.60	0.59	0.34	0.26
Nitrate & Nitrite (N)	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05
Ammonia (N)	0.10	<0.05	<0.29	<0.05	0.07	<0.05
Arsenic	1.7*	12.5*	9.6*	1.1*	3.4*	2.9*
Iron	0.15	45.0*	19.0*	0.07	15.0*	12.0*
Manganese	3.9*	7.4*	5.9*	0.39*	1.8*	1.4*
Copper	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Zinc	0.01	0.01	<0.01	0.01	<0.01	<0.01
Colour (T.C.V)	17.0*	–	330.0*	10.0	–	10.0
Conductivity (micromho/cm)	545.00	920.0	570.0	386.0	445.0	431.0
pH (unit)	7.20	6.80	6.80	7.20	6.90	7.10
Lead-ICP & HGA	<0.05	<0.002	<0.002	<0.05	<0.002	<0.002
Aluminum	<0.05	<0.05	<0.05	<0.05	0.05*	<0.05
Boron	<0.02	<0.02	<0.02	<0.02	<0.02	<0.02
Barium	0.065	0.107	0.081	0.037	0.030	0.031
Beryllium	<0.005	<0.005	<0.005	<0.005	<0.005	<0.005
Chromium	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Cobalt	<0.01	<0.01	–	<0.01	<0.01	–
Nickel	<0.02	<0.02	0.02	<0.02	<0.02	<0.02
Antimony	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05
Selenium	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
Tin	0.06	<0.03	0.05	0.06	<0.03	0.04
Vanadium	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Cadmium	<0.01	–	<0.01	<0.01	<0.01	<0.01
Mercury (ppm)	–	<0.05	<0.05	–	<0.05	<0.05
Sodium	–	8.6	–	–	3.8	–
Potassium	–	8.9	–	–	2.2	–
Total organic carbon	–	18.2	–	–	4.5	–

*Values exceeding recommended concentrations are marked with an asterisk. All values are in mg/L unless otherwise stated. Locations of piezometers (P1–P6) are presented in Figure 1.

Table 2 – Fluctuations in water table depth in Oldham tailings

Date	Water table depth (cm)															
	June			July			August						September			
	3	10	24	20	26	27	2	3	9	12	16	17	6	10	20	2
Piezometer																
1	34	20	38	39	2	7	5	10	23	28	23	20	24	31	–	21
2	40	14	50	61	7	11	10	16	35	36	28	26	36	37	31	30
3	30	25	31	40	8	16	15	19	26	25	12	15	–	–	16	18
4	26	25	34	37	6	6	7	13	24	26	20	21	27	20	21	–
5	13	−2	9	11	−7	−6	−6	−6	−4	−1	−3	−4	−3	−2	–	−3
6	28	13	30	39	1	2	6	9	14	24	11	17	19	25	15	18

Table 3 – Water quality data for surface waters entering and leaving the Oldham tailings deposit*

	Site 1 Upstream of tailings			Site 2 Midway through tailings		
Date	3/6/88	12/8/88	5/11/88	3/6/88	12/8/88	5/11/88
Calcium	1.7	1.9		2.7	2.6	
Magnesium	0.49	0.60		0.55	0.64	
Hardness ($CaCo_3$)	6.3	7.3		9.0	9.0	
Alkalinity ($CaCo_3$)	<1.0	<1.0		0.90	<1.0	
Sulphate	7.3	<2.0		7.7	<2.0	
Chloride	3.2	3.3		4.2	3.61	
Ortho–Phosphorus (P)	<0.01	<0.01		0.01	0.01	
Nitrate & Nitrite (N)	0.53	<0.05		0.16	<0.05	
Ammonia (N)	0.44	0.18		0.28	0.17	
Arsenic	<0.005	<0.005	0.020	0.060*	0.130*	0.040
Iron	1.2*	3.2*		0.99*	3.5*	
Manganese	0.08*	0.10*		0.14*	0.18*	
Copper	0.01*	<0.01		<0.01	<0.01	
Zinc	0.01	0.01		0.01	0.01	
Colour (T.C.U)		96.0*	81.0*		85.08	190.0*
Conductivity (micromho/cm)	55.40	54.4		43.9	48.2	
pH (units)	4.30*	4.30*		5.50*	4.60*	
Lead–ICP & HGA	<0.05	<0.002		<0.05	<0.002	
Aluminum	<0.05	0.59*		0.37*	0.58*	
Boron	<0.02	<0.02		<0.02	<0.02	
Barium	<0.005	0.005		<0.005	0.005	
Beryllium	<0.005	<0.005		<0.005	<0.005	
Chromium	0.02*	<0.01		<0.01	<0.01	
Cobalt	<0.01	–		<0.01	–	
Nickel	<0.02	<0.02		<0.02	<0.02	
Antimony	<0.05	<0.05		<0.05	<0.05	
Selenium	<0.1	<0.1		<0.1	<0.1	
Tin	<0.03	<0.03		<0.03	<0.03	
Vanadium	<0.01	<0.01		<0.01	<0.01	
Cadmium	<0.01	<0.01		<0.01	<0.01	
Mercury (ppm)	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05
Turbidity (J.T.U.)	1.8	–		1.8	–	
Acidity	12.0	–		8.3	–	

*Values exceeding recommended concentrations are marked with an asterisk. All values are in mg/L unless otherwise stated. Locations of sampling sites are presented in Figure 1.

Table 3 (Cont'd)

	Site 3 Downstream of tailings			Site 4 Upstream of pond		Site 5 Pond
Date	3/6/88	12/8/88	5/11/88	3/6/88	12/8/88	5/11/88
Calcium	3.5	2.9	8.9	14.0	7.0	15.0
Magnesium	0.61	0.67	0.71	1.1	0.79	1.0
Hardness ($CaCo_3$)	11.2	9.9	25.2	39.4	20.7	41.7
Alkalinity ($CaCo_3$)	3.1	<1.0	24.0	39.0	20.0	41.0
Sulphate	7.3	<2.0	4.2	<2.0	3.0	<2.0
Chloride	4.0	3.8	1.8	2.1	1.3	2.1
Ortho-Phosphorus (P)	0.02	0.01	<0.01	0.01	0.02	0.03
Nitrate & Nitrite (N)	0.13	<0.05	<0.05	0.10	<0.05	<0.05
Ammonia (N)	0.24	0.17	<0.05	<0.05	<0.05	<0.05
Arsenic	0.070*	0.150*	0.020	0.040	0.060*	0.100*
Iron	0.98*	4.7*	0.09	0.06	0.64*	0.93*
Manganese	0.15*	0.21*	<0.01	0.02	0.03	0.19*
Copper	<0.01*	<0.01	<0.01	<0.01	<0.01	0.01*
Zinc	–	0.01	<0.01	<0.01	<0.01	<0.01
Colour (T.C.U)	85.0*	190.0*	15.0	22.0*	29.0*	41.0*
Conductivity (micromho/cm)	44.20	47.2	62.6	90.2	51.3	89.6
pH (units)	6.30*	4.70*	7.40	7.20	7.60	7.30
Lead-ICP & HGA	<0.05	<0.002	<0.05	<0.002	<0.05	<0.002
Aluminum	0.30*	0.67*	0.08	<0.05	<0.05	<0.05
Boron	<0.02	<0.02	<0.02	<0.02	<0.02	0.02
Barium	0.005	0.006	<0.005	0.006	<0.005	<0.005
Beryllium	<0.005	<0.005	<0.005	<0.005	<0.005	<0.005
Chromium	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Cobalt	<0.01	–	–	–	<0.01	–
Nickel	<0.02	<0.02	<0.02	<0.02	<0.02	<0.02
Antimony	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05
Selenium	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
Tin	<0.03	<0.03	<0.03	<0.03	<0.03	0.03
Vanadium	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Cadmium	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Mercury (ppm)	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05
Turbidity (J.T.U)	1.8	–	0.3	–	1.4	–
Acidity	6.7	–	–	–	–	–

Table 4 – List of species noted during the vegetations survey on the Oldham tailings deposit

Binomial	Common name
Abies balsamea	balsam fir
Acer rubrum	red maple
Agrostis palustris	creeping bent–grass
Agrostis perennans	bentgrass
Agrostis scabra	tickle–grass
Alnus rugosa	speckled alder
Amelanchier sp.	shadbush
Aronia arbutifolia	red chokeberry
Aster lateriflorus	calico aster
Aster nemoralis	bog aster
Aster novi–belgii	New York aster
Aster umbellatus	tall white aster
Calamagrostis canadensis	blue–joint
Campylium stellata	
Carex brunesscens	sedge
Carex leptalia	sedge
Carex nigra	sedge
Carex rostrata	sedge
Carex stipata	sedge
Carex tenera	sedge
Carex viridula	sedge
Centaurea nigra	knapweed
Chamaedaphne calyculata	leatherleaf
Chara globularis	
Chrysanthemum leucanthemum	ox–eye–daisy
Cinna latifolia	wood–reed
Drosera intermedia	narrow–leaved sundew
Drosera rotundifolia	round–leaved sundew
Dryopteris thelypteris	bog fern
Equisetum arvense	field horsetail
Equisetum fluviatile	water horsetail
Equisetum variegatum	horsetail
Eupatorium perfoliatum	boneset
Festuca rubra	red fescue
Fragaria virginiana	wild strawberry
Glyceria fernaldii	small manna–grass
Juncus articulatus	rush
Juncus effusus	soft rush
Juncus pelocarpus	rush

Table 4 (Cont'd)

Binomial	Common name
Larix laricina	larch
Leontodon autumnalis	fall dandelion
Linaria vulgaris	butter–and–eggs
Lonicera villosa	mountain fly–honeysuckle
Lycopus americanus	water–horehound
Lycopus uniflorus	bugle weed
Lysimachia terrestris	loosestrife
Mentha arvense	field mint
Muhlenbergia uniflora	
Myosotis sp.	forget–me–not
Myrica gale	sweet gale
Onoclea sensibilis	sensitive fern
Osmunda regalis	royal fern
Picea glauca	white spruce
Picea rubens	red spruce
Plantago major	broad–leaved plantain
Polytricum commune	hair–cap moss
Prunus virginiana	choke cherry
Prunella vulgaris	heal–all
Ranunculus repens	creeping buttercup
Rhinanthus crista–galli	yellow rattle
Rosa carolina	wild rose
Rubus hispidus	trailing blackberry
Rubus pubescens	dewberry
Rubus vermontanus	blackberry
Salix humilis	small pussy–willow
Scirpus rubrotinctus	bulrush
Solidago canadensis	Canada goldenrod
Solidago rugosa	rough goldenrod
Sphagnum sp.	sphagnum moss
Spiranthes cernua	nodding ladies'–tresses
Spiraea latifolia	hardhack
Thalictrum polygamum	meadow–rue
Typha latifolia	broad–leaved cattail
Utricularia intermedia	bladderwort
Viburnum cassinoides	witherod
Viola cucullata	blue violet
Viola pallens	small white violet

Table 5 – Concentrations of arsenic and mercury in shoots and roots of plant species associated with tailings deposits at Oldham.

	Arsenic (ppm)		Mercury (ppm)	
	Shoots	Roots	Shoots	Roots
Microspora quadrata	2840	–	3.19	–
M. quadrata uncontaminated	13	–	–	–
Equisetum fluviatile	700	3050	0.45	0.47
E. fluviatile uncontaminated*	2	–	–	–
Juncus articulatus	580	6340	0.55	2.69
Juncus pelocarpus	330	5620	0.54	6.11
Dulichium arundinaceum	400	3110	0.19	0.67
Sparganium fluctuans	4260	–	16.3	–
Leersia oryzoides	321	2650	0.18	1.56

*From Dale and Freedman, 1982

Table 6 – Species biomass and frequency of occurrence for all common species in each of seven plant communities*

Species	Plant community							
	Cluster 1 C.g.–E.f.		Cluster 2 J.a.–E.f.		Cluster 3 C.s.–E.f.		Cluster 4 E.f.–A.p.	
	Biomass g/m²(SE)	Frequency (%)	Biomass g/m²(SE)	Frequency (%)	Biomass g/m²(SE)	Frequency (%)	Biomass g/m²(SE)	Frequency (%)
Acer rubrum	–	–	–	–	–	–	–	–
Agrostis palustris	–	–	3.88(220)	67	14.0(2.86)	95	25.5(6.50)	100
Alnus rugosa	–	–	–	–	–	–	–	–
Aster novi-belgii	–	–	2.65(1.27)	42	1.11(0.29)	73	6.98(2.69)	73
Aster umbellatus	–	–	–	–	–	–	–	–
Campylium stellata	–	–	2.16(1.61)	13	191(40.2)	91	4.87(2.29)	40
Carex brunescens	–	–	0.39(0.36)	13	–	–	–	–
Centaurea nigra	–	–	0.02(0.02)	13	2.48(0.97)	36	2.54(1.54)	40
Chara globularis	99.6(9.72)	100	0.92(0.67)	8	–	–	–	–
Chrysanthemum leucanthemum	–	–	0.18(0.12)	17	1.92(0.72)	36	0.48(0.35)	27
Equisetum arvense	–	–	1.27(0.82)	21	0.22(0.16)	14	31.4(10.4)	67
Equisetum fluviatile	14.0(7.34)	67	35.5(6.19)	100	50.4(5.01)	95	51.3(13.0)	80
Equisetum variegatum	–	–	0.22(0.15)	17	9.05(2.69)	59	2.43(1.36)	47
Eupatorium perfoliatum	–	–	20.1(13.3)	29	5.09(1.85)	55	2.19(12.5)	67
Hypericum virginicum	–	–	0.83(0.44)	50	0.49(0.41)	18	0.73(0.43)	27
Juncus articulatus	–	–	101(18.4)	92	2.93(1.21)	45	1.53(0.76)	53
Juncus effusus	–	–	–	–	–	–	–	–
Lycopus uniflorus	–	–	0.64(0.27)	58	1.63(0.50)	59	0.71(0.31)	47
Lysimachia terrestris	–	–	1.82(0.58)	58	0.38(0.20)	18	1.24(0.45)	53
Onoclea sensibilis	–	–	0.22(0.22)	8	1.30(0.69)	14	0.45(0.45)	7
Polytrichum commune	–	–	–	–	–	–	–	–
Prunella vulgaris	–	–	0.54(0.24)	38	3.15(1.11)	59	0.83(0.27)	60
Rubus pubescens	–	–	–	–	–	–	–	–
Spiraea latifolia	–	–	–	–	–	–	–	–
Utricularia intermedia	8.79(2.46)	100	0.04(0.04)	8	–	–	–	–
Viola cucullata	–	–	0.31(0.20)	21	–	–	0.33(0.17)	27
Viola pallens	–	–	0.33(0.23)	33	0.21(0.12)	41	0.55(0.25)	60
Total biomass g/m²	122(12.3)		188(26.4)		297(39.0)		171(20.4)	

*Plant community codes are as follows: C.g.–E.f. = *Chara globularis–Equisetum fluviatile* community, J.a.–E.f. = *Juncus articulatus–E. fluviatile* community, C.s.–E.f. = *Campylium stellata–E. fluviatile* community, E.f.–A.p. = *E. fluviatile*–Agrostis *palustris* community, E.f.–E.p.= *E. fluviatile–Eupatorium perfoliatum* community, Short A.r.–S.l.= Short *Alnus rugosa–Spiraea latifolia* community and Tall A.r.–S.l. = Tall *A. rugosa–S. latifolia* community. Mean total biomass for each community, is also presented.

Table 6 (Cont'd)

Species	Plant community					
	Cluster 6 E.f.–E.p.		Cluster 7 Short A.r.–S.l.		Cluster 5 Tall A.r.–S.l.	
	Biomass g/m²(SE)	Frequency (%)	Biomass g/m²(SE)	Frequency (%)	Biomass g/m²(SE)	Frequency (%)
Acer rubrum	–	–	50.9(383)	35	0.76(0.51)	24
Agrostis palustris	12.0(4.36)	63	2.27(1.68)	24	0.18(0.17)	100
Alnus rugosa	–	–	828(190)	100	720(1540)	100
Aster novi–belgii	3.50(1.42)	53	3.02(2.29)	18	0.77(0.76)	12
Aster umbellatus	9.66(8.52)	21	19.2(7.31)	71	9.71(3.48)	71
Campylium stellata	37.2(10.4)	58	7.57(3.51)	41	1.86(1.47)	12
Carex brunessens	–	–	8.97(6.35)	35	–	–
Centaurea nigra	0.22(0.16)	11	0.04(0.04)	6	0.26(0.19)	12
Chara globularis	–	–	–	–	–	–
Chrysanthemum leucanthemum	0.69(0.42)	21	–	–	–	–
Equisetum arvense	28.5(6.39)	79	4.44(2.55)	35	8.07(2.43)	71
Equisetum fluviatile	29.7(9.96)	89	6.39(2.01)	82	1.50(0.67)	71
Equisetum variegatum	3.41(1.48)	32	–	–	0.03(0.02)	12
Eupatorium perfoliatum	58.6(23.6)	58	5.72(2.77)	29	–	–
Hypericum virginicum	11.5(6.11)	32	3.60(2.77)	53		
Juncus articulatus	5.13(2.63)	37	8.86(5.72)	24	0.02(0.02)	6
Juncus effusus	15.9(11.1)	16	11.3(5.91)	41	0.07(0.07)	6
Lycopus uniflorus	1.90(1.38)	42	0.39(0.21)	41	0.99(0.40)	76
Lysimachia terrestris	1.45(0.68)	47	2.20(1.46)	41	0.52(0.26)	29
Onoclea sensibilig	0.28(0.28)	5	3.33(2.18)	24	4.41(3.33)	47
Polytrichum commune	16.5(7.26)	32	18.2(14.9)	29	0.08(0.08)	6
Prunella vulgaris	1.91(1.30)	21	–	–	–	–
Rubus pubescens	0.35(0.35)	5	0.44(0.25)	24	3.76(1.46)	53
Spiraea latifolia	45.6(37.6)	11	179(45.4)	88	104.0(33.8)	94
Utricularia intermedia	–	–	–	–	–	–
Viola cucullata	0.44(0.18)	47	0.28(0.18)	41	0.15(0.09)	18
Viola pallens	0.86(0.32)	53	0.31(0.21)	65	0.13(0.09)	41
Total biomass g/m²	324(36.5)		1350(188)		4960(1530)	

Table 7 – Mean values of depth to water table, thickness of organic matter, per cent organic matter and arsenic and mercury concentrations in the upper 10 cm of the soil for each of seven plant communities*

	Cluster 1 Cg–E.f.	Cluster 2 J.a.–E.f.	Cluster 3 C.s.–E.f.	Cluster 4 E.f.–A.p.	Cluster 6 E.f.–E.p.	Cluster 7 Short Ar.–S.l.	Cluster 5 Tall A.r.–S.l.
Mean depth of water table [cm (SE)]	−35.3(1.73)	0.28(2.34)	13.3(1.70)	18.4(2.84)	21.8(3.87)	10.8(2.27)	19.5(3.14)
Mean thickness of organic layer [cm (SE)]	1.33(0.42)	5.12(0.67)	1.95(0.20)	2.93(0.66)	5.79(0.98)	8.41(0.59)	6.82(0.65)
Mean per cent organic matter [cm (SE)]	4.70(1.54)	4.70(0.90)	1.19(0.12)	4.59(2.15)	8.32(2.01)	30.3(4.66)	17.2(3.96)
Mean soil arsenic concentration [ppm (SE)]	263(238)	893(178)	829(238)	578(298)	4430(2740)	1470(974)	1240(528)
Mean soil mercury concentration [ppm (SE)]	6.15(0.76)	4.25(0.48)	4.49(0.65)	3.92(0.02)	6.41(1.10)	3.61(0.62)	4.85(1.17)

*Plant community codes are as follows: Cg–E.f. = *Chara globularis–Equisetum fluviatile* community, J.a.–E.f. = *Juncus articulatus–E. fluviatile* community, C.s.–E.f. = *Campylium stellata–E. fluviatile* community E.f.–A.p.=*E. fluviatile–Agrostis palustris* community, E.f.–E.p.= *E. fluviatile–Eupatorium perfoliatum* community, Short A.r.–S.l. = Short *Alnus rugosa–Spiraea latifolia* community and Tall A.r.–S.l. = Tall *A. rugosa–S. latifolia* community.

Table 8 – Site water status and soil organic matter content preferences for species native to the Oldham tailings

Species	Site water status	Soil organic matter content
Agrostis palustris	xeric	low
Alnus rugosa	mesic	high
Aster novi–belgii	xeric	low
Aster umbellatus	hydric	high
Campylium stellata	mesic	low
Centaurea nigra	xeric	low
Chara globularis	hydric	low
Chrysanthemum leucanthemum	mesic	low
Equisetum arvense	xeric	low
Equisetum fluviatile	mesic	low
Equisetum variegatum	mesic	low
Eupatorium perfoliatum	mesic	moderate
Hypericum virginicum	hydric	high
Juncus articulatus	hydric	moderate
Juncus effusus	mesic	moderate
Lycopus uniflorus	mesic	low
Lysimachia terrestris	hydric	moderate
Onoclea sensibilis	mesic	high
Polytrichum commune	mesic	moderate
Prunella vulgaris	mesic	low
Rubus pubescens	mesic	high
Spiraea latifolia	mesic	high
Utricularia intermedia	hydric	low
Viola cucullata	mesic	moderate
Viola pallens	mesic	moderate

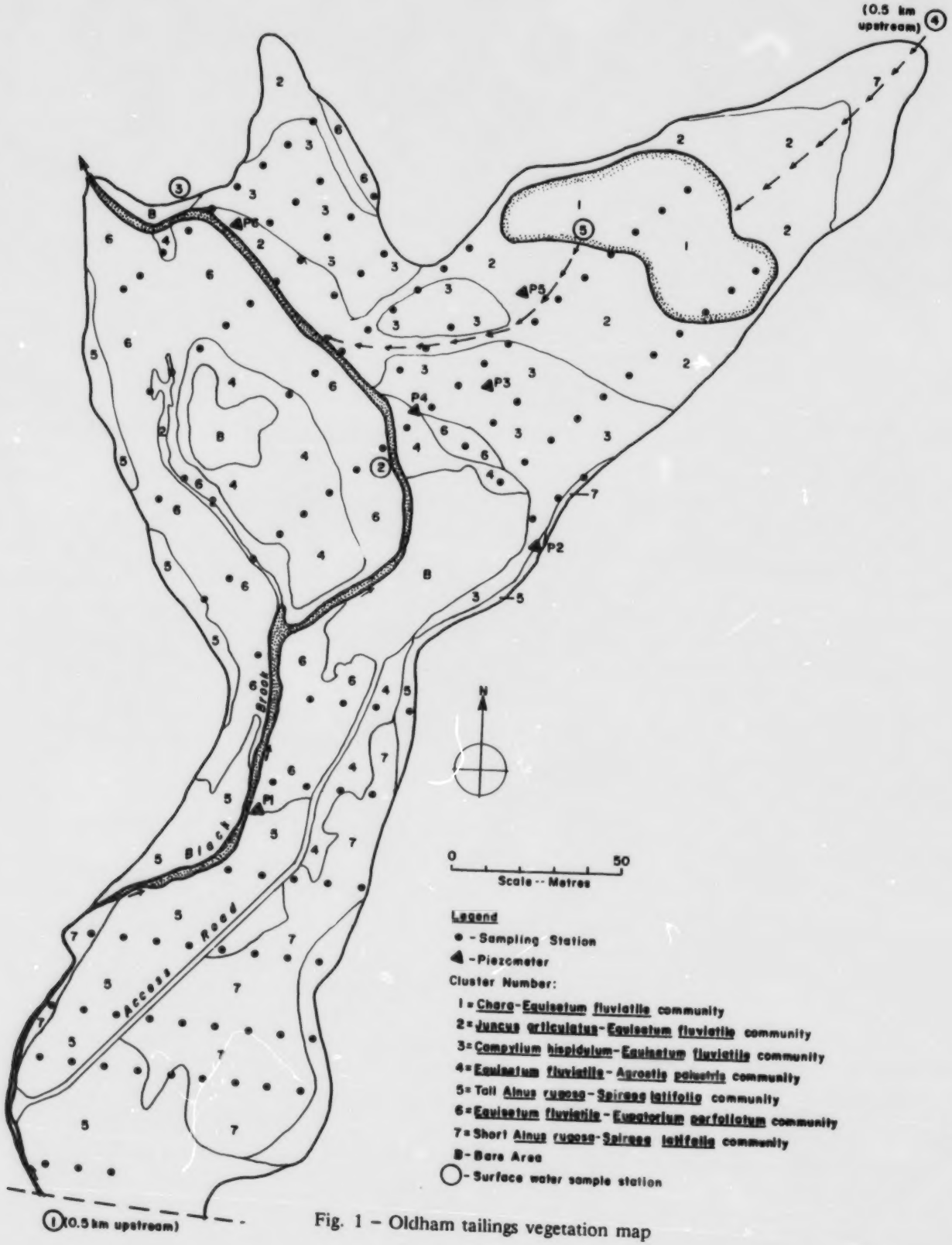

Fig. 1 – Oldham tailings vegetation map

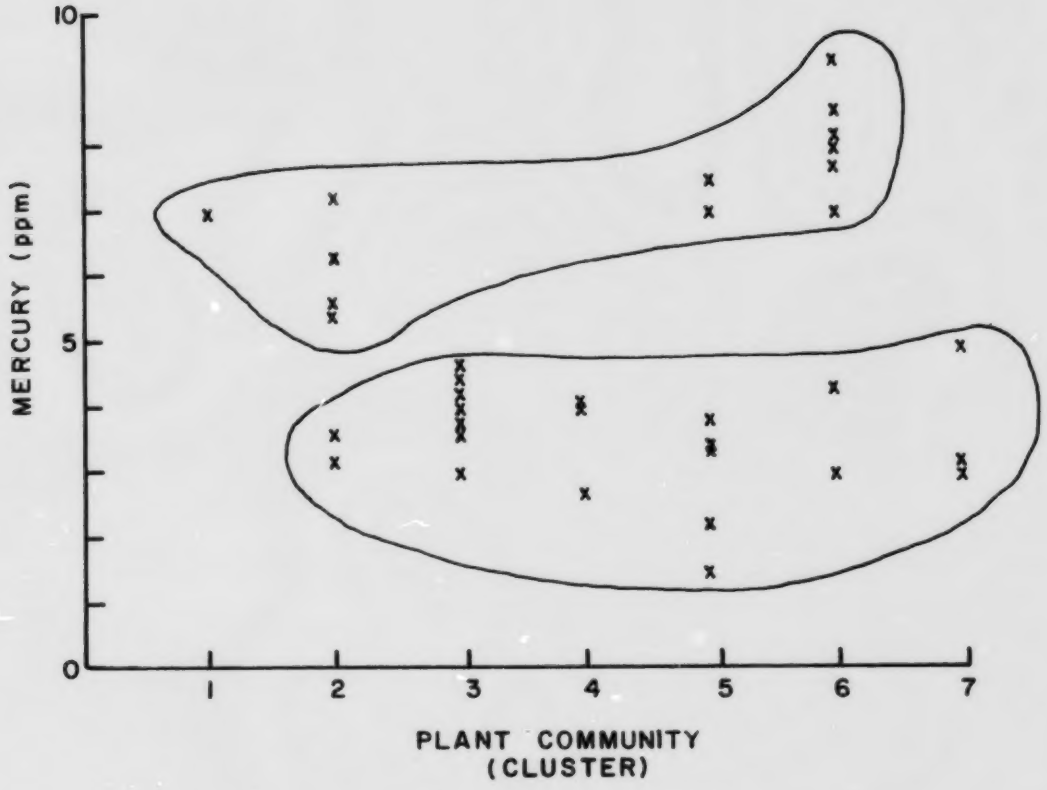

Fig. 2 – Preferential enrichment of Hg in the upper 10 cm of tailings in wet plant communities

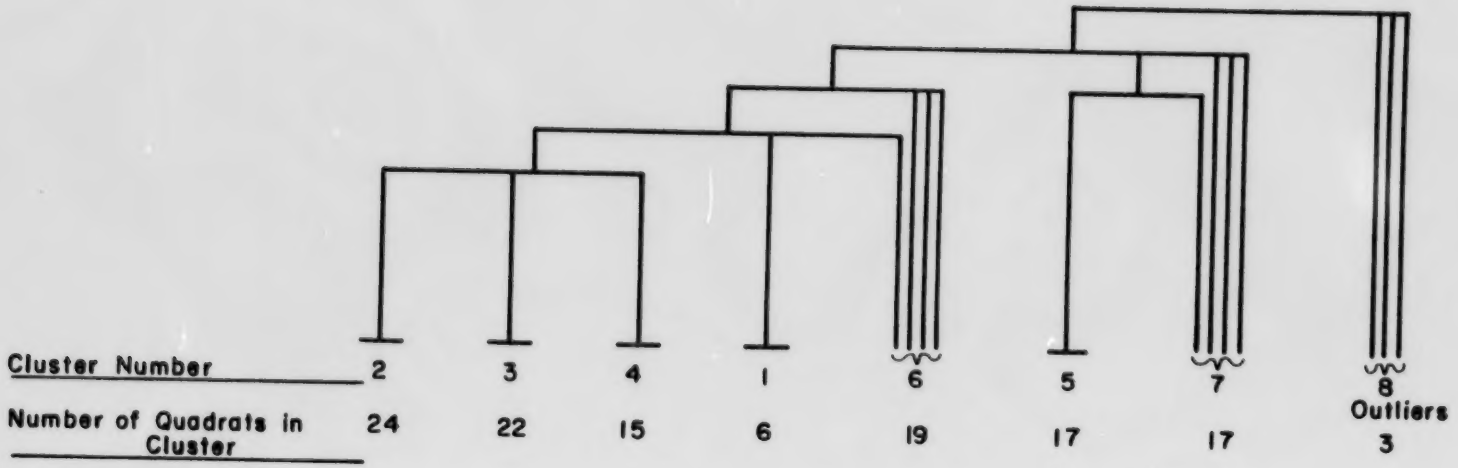

Fig. 3 – Schematic of cluster diagram

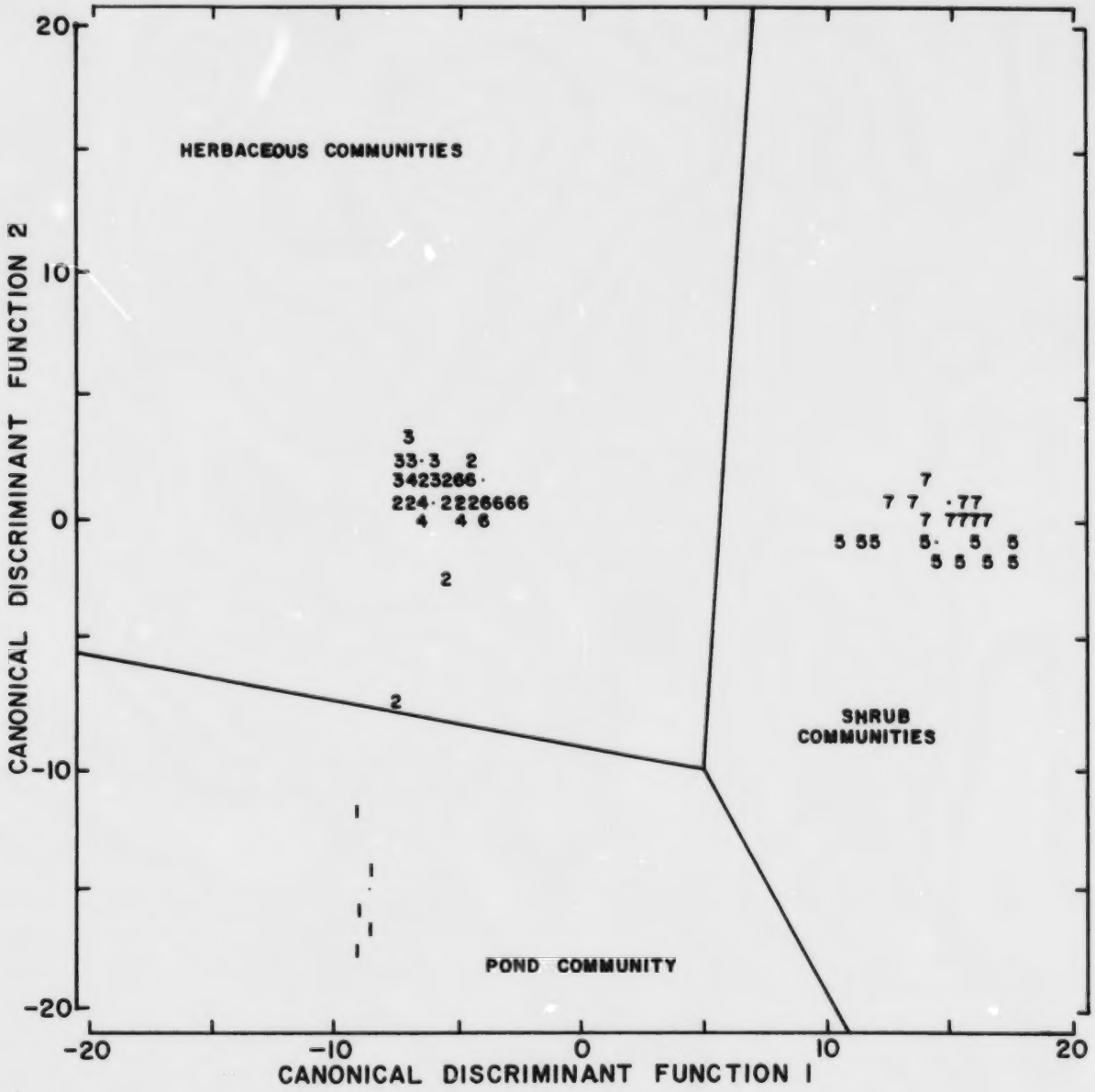

Fig. 4 – Scatterplot of site and community location on the first two discriminant function axes, using community species compositions as the discriminating variables

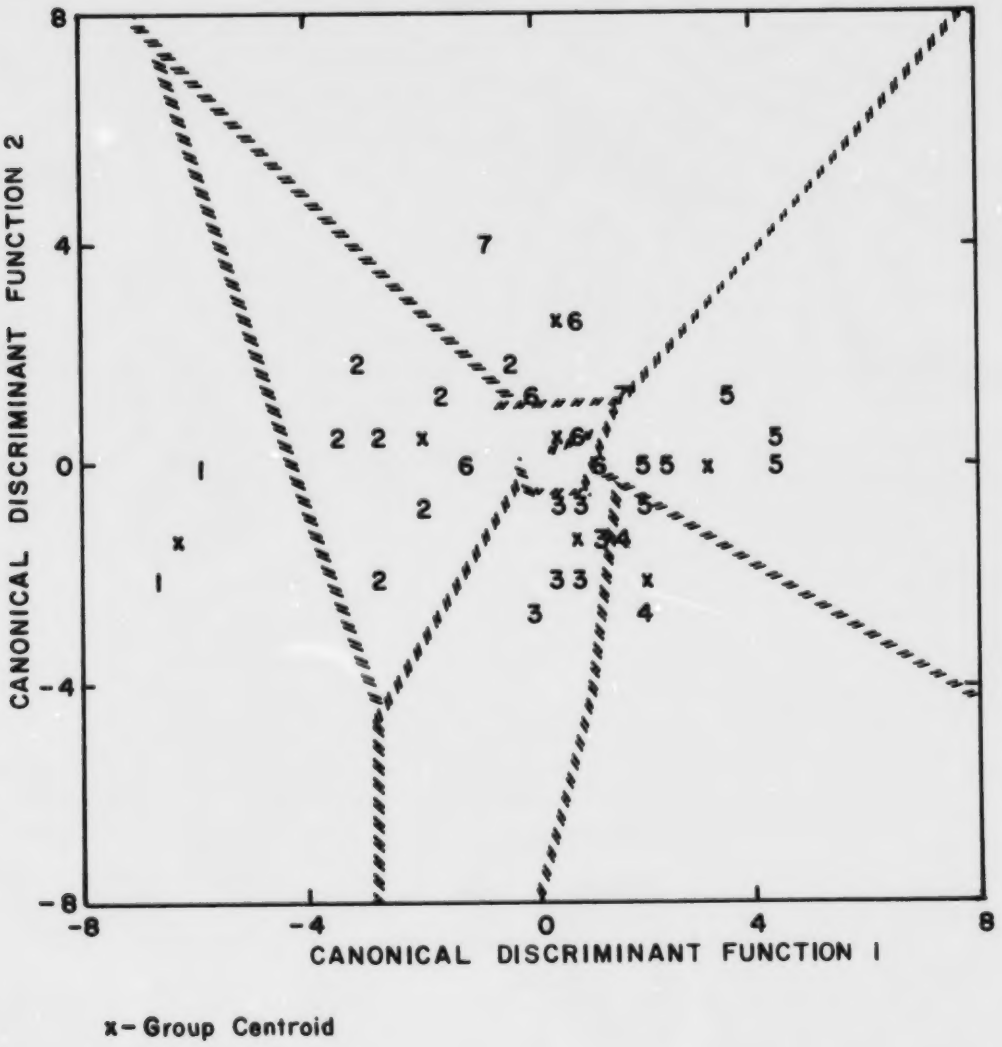

Fig. 5 – Scatterplot of site and community location on the first two discriminant function axes, using features of the physical environment as the discriminating variables

SESSION I: PAPER 2

MICROBIAL TREATMENT OF INDUSTRIAL EFFLUENTS

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ABSTRACT

Over the past few years CANMET has developed several biological processes for the treatment of industrial effluents based on rotary biological contactors (RBC). Discussion will include the recovery of elemental selenium from solution by bioreduction, biodegradation of an organic acid from a mill process stream, biodegradation of airport runoffs containing de-icing fluids and urea and the potential application of this technology to remove organics from process waters to allow their recycle into the process circuit. Biosorption of uranium and radium will also be discussed.

Keywords: acid mine drainage; mine effluents; metal adsorption; metal reduction; rotating biological contactors; ethylene glycol

SESSION I : EXPOSÉ 2

TRAITEMENT MICROBIEN D'EFFLUENTS INDUSTRIELS

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RÉSUMÉ

Au cours des quelques dernières années, CANMET a mis au point plusieurs procédés biologiques de traitement d'effluents industriels au moyen de contacteurs biologiques rotatifs (RBC). La discussion traite, entre autres, de la récupération de sélénium métallique dans une solution par bioréduction, de la biodégradation d'un acide organique obtenu à partir d'un circuit de broyage, de la biodégradation des eaux de ruissellement d'un aéroport – qui contiennent des liquides à dégivrer et de l'urée – ainsi que de l'application potentielle de cette technologie pour l'élimination de substances organiques dans des eaux de fabrication de façon à permettre leur recyclage dans le circuit de traitement. La biosorption de l'uranium et du radium sera aussi traitée.

Mots-clé : neutralisation des eaux de drainage dans l'environnement minier; effluents industriels; adsorption des métaux; contacteurs biologiques rotatifs (RBC); éthylène glycol.

MICROBIAL TREATMENT OF INDUSTRIAL EFFLUENTS

MINE WATER CHEMISTRY AND CURRENT TECHNOLOGY

BASE METAL MINES

Mines processing copper (Cu), zinc (Zn) and lead (Pb) from sulphide ores produce acidic mine drainage from their tailings piles due to chemical and biological oxidation of the pyrrhotite and pyrite associated with the mineral sulphides. The chemistry of such tailings effluents are presented in Table 1. The concentration of each ion varies considerably depending on the mineralogy of the ore, efficiency of processing and the environmental conditions at the mine site.

Acidic drainage from base metal mines is generally collected and treated by the addition of a limestone slurry to neutralize the sulphuric acid and precipitate the metal ions as hydroxides.

URANIUM MINES

Processing of pitchblend deposits produces non–acid generating tailings. However, these tailings generally release radium, arsenic and nickel. The radium and nickel are removed by barium sulphate co–precipitation, and the arsenic is removed by precipitation with iron.

In contrast, sulphidic uranium ores, such as those found in the Elliot Lake area, contain 3–5% pyrite and the tailings are acid generating.

The water chemistry of the uranium tailings acidic drainage is presented in Table 2.

Uranium tailings effluents are generally treated by the addition of barium to co–precipitate the radium as $Ba/RaSO_4$, and the pH is adjusted by the addition of a limestone slurry which effectively precipitates the metal ions as hydroxides.

GOLD MINES

Gold tailings are generally quite alkaline because they are processed with alkaline cyanide for gold recovery. However, refractory gold ore tailings in which the gold is associated with arsenopyrite can, over a period of time, become acidic. The major pollutants in gold mill effluents are free cyanide and cyanide complexes (Table 3).

Currently, gold mill effluents are treated by one of four treatment schemes to reduce the cyanide levels to environmental regulatory concentrations (2 mg CN_T/L; Ontario Regulations).

Cyanide will degrade naturally in the environment by a combination of photolysis, volatilization and microbial degradation. This process is very effective during the summer months in Canada, but cyanide levels are quite high during the winter months. Data from three different mines utilizing natural degradation in treatment lagoons are presented in Figure 1.

Chemical treatment of cyanide effluents include: (i) oxidation with H_2O_2; (ii) the Inco SO_2–Air treatment process; and (iii) alkaline chlorination. The last process is very expensive and is not effective for arsenic–containing effluents.

Imperial Chemical Industries are attempting to market a lyophilized fungus preparation for the degradation of cyanide in effluent. The cells produce cyanide hydratase which degrades CN^- to formamide, and which can subsequently be metabolized by other microorganisms to CO_2 plus ammonia. The preparation, however, loses half of its enzyme activity in 20 h and does not degrade metal cyanide complexes.

Recently, CANMET has developed the AVR process (Acid Volatilization and Recovery process) and recycling of 97% of the cyanide can be achieved.

BIOLOGICAL PROCESS DEVELOPMENT FOR MINE AND MILL EFFLUENTS

Biorecovery of Selenium from Roaster and Smelter Weak Acid Effluents

McCready et al., 1966 showed that the reduction of selenite occurred via two 2–electron transfer steps – $Se^{4+} \rightarrow Se^{2+} \rightarrow Se^0$ – and the reduction occurred at different rates depending on the organism used to mediate the reaction (see Table 4).

These authors concluded that since sulphur and selenium are both in group 6A of the periodic table, and since they have similar chemical and physical properties, the reductive enzyme may be the same or similar in the two systems. S. heidelberg reduces Na_2SO_3 to H_2S. Both SO_3 and SeO_3 pass through the same valence states on reduction; however, the difference in the end product valence state may be due to the great differences in the oxidization–reduction potentials of the two systems. The following represents the oxidation–reduction of the two systems:

$$\left.\begin{matrix} H_2SO_3 \\ S^{+2} \end{matrix}\right\} \quad 0.41V \qquad\qquad 0.74V \quad \left\{\begin{matrix} H_2SeO_3 \\ Se^{+2} \end{matrix}\right.$$

$$\left.\begin{matrix} [S^0] \\ H_2S_{(aq)} \end{matrix}\right\} \quad 0.141V \qquad\qquad -0.35V \quad \left\{\begin{matrix} [Se^0] \\ H_2Se_{(aq)} \end{matrix}\right.$$

In the sequential reduction of SO_3^{2-} elemental S^0 is not released as is Se^0, but may remain organically bound and further reduced to S^{2-}.

Using this background information, a process design based on a rotary biological contactor was developed for the removal of selenium salts from mine effluents and was recently pilot tested by CMS Rotodisk in Mississauga.

LABORATORY STUDIES

Selenite reducing organisms were grown in Trypticase Soy Broth (Baltimore Biological Co. Ltd.) containing 0.1% w/v SeO_3^{2-} (456 ppm Se) at 37°C. The growth, changes in pH and deposition of intracellular selenium granules were followed over a 32–h period. The organisms were capable of reducing selenite to elemental selenium over the pH range of 6.0 to 8.5 with an optimal rate of reduction at pH 7.0 (see Fig. 2–5).

Simultaneously, the cultures were examined and photographed under phase contrast microscope at various timed intervals. Granule deposition was observed from 4 h to 12 h after inoculation. When

the selenite concentration had been substantially reduced, normal logarithmic growth of the cultures was observed (Fig. 6).

PILOT-PLANT STUDIES

Under the NRC-IRAP program, CMS Rotodisk Inc. conducted a pilot-plant study for the removal of selenium from a weak acid effluent discharged from a base metal smelter. A laboratory scale rotary biological contractor with 100 ft^2 of disk surface and a 30-L capacity was used for the study (Fig. 7).

The weak acid effluent was neutralized to pH 7.0 and was combined with the microbial inoculum and a nutrient solution as an input stream to the rotary biological contactor (RBC). Tests were conducted to optimize parameters such as C:Se ratio, flow rate and retention time.

Using the RBC, more than 97% of the selenium in the weak acid effluent is removed, with a retention time of 4 h. The selenium laden biomass that sloughs off the disks of the RBC is recovered from the RBC effluent by centrifugation.

As the selenium granules are within the cytoplasm of the bacterial cells, the cell walls and membranes must be ruptured in order to recover the metal.

CANMET is currently developing a process for the recovery of the selenium granules from the biomass. Tests, including methanol:KOH extraction, and the use of lytic enzymes such as lipase and protease, are being conducted on various procedures to rupture cell walls and membranes. To date, the recovered selenium has been about 99% purity with 0.02% sulphur and traces of potassium, and the balance is presumed to be cellular carbohydrates or protein (see Table 5). Tests are being conducted to remove the contaminating materials while keeping the number of steps and the cost of the process to a minimum.

From the pilot-plant study and the selenium recovery project it has been estimated that the capital cost of treating 1100 gal/m of effluent at an average selenium content of 40 ppm would be about 3.4×10^6 with an annual operating cost of $90,000. The potential return is 2×10^6 worth of selenium per year at the current market price of $6 per pound.

Bioadsorption of Radium

Large quantities of radioactive $Ba/RaSO_4$ sludge are produced annually at water treatment plants in the uranium industry. Disposal of these sludges on mine close-out will prove to be difficult. If sludges are capped with clay and become anaerobic, there is the potential for sulphate-reducing organisms (Desulphovibrio, Desulphotomaculum or Clostridia) to attack the sludges and remobilize both the Ba and Ra into the groundwater (McCready et al., 1980).

One possible alternative is the bioadsorption process for radium removal from solution developed by Dr. Marios Tsezos at McMaster University. Dr. Tsezos has shown that radium can be adsorbed from uranium mine effluents by using immobilized and pelletized sewage sludge. The pellets developed to date cannot be stripped of radium and recycled. One can envisage using the bioadsorbant to trap the radium, sealing the radium-saturated pellets in closed canisters and disposing of them at a low-level radiation disposal site.

Bioadsorption of Uranium from Bacterial Leach Solutions

CANMET has developed a bioadsorption process for the recovery of uranium from the dilute (250–300 mg U/L) leach solutions being produced at Denison Mines during their bacterial leaching of uranium process.

Using pelletized, immobilized *Rhizopus arrhizus* in an up–flow column design (Fig. 8), McMaster University recently completed twelve loading and stripping cycles for the adsorption of uranium (Table 6). Thirty–fold concentration of the uranium has been achieved and a larger pilot–plant study will be conducted this year to assess the life of the biomass pellets, to optimize the flow rate and to obtain sufficient data to conduct an economic assessment of the process.

Biodegradation of an Organic Pollutant

During certain mineral processing operations using harsh extraction procedures, organic components of the ore are degraded to toxic components. CANMET, in cooperation with a major mining company, has developed an RBC–based process for the degradation of the organic pollutant to CO_2 and H_2O.

Ninety–nine per cent of the pollutant is degraded within the RBC with an influent concentration of 12 g/L and a 5–h retention time. This process may be tested industrially in the coming year.

Microbial Degradation of Ethylene Glycol

During the winter, airport runoff contains urea, ethylene glycol and/or propylene glycol from de–icing operations. The high biological oxygen demand (BOD) of the airport runoff causes problems in the municipal waste water treatment facilities. Thus, it is desirable that most of the glycol be degraded before the runoff can be released to surface waters.

Samples for the isolation of ethylene glycol degrading bacteria were obtained from two sources: (1) an aerobic lagoon at the Calgary International Airport that is used for the treatment of de–icing fluids in the airport runoff, and (2) soil samples from several locations adjacent to taxiways Alpha and Bravo at the Ottawa International Airport.

Either 5 mL of water (Calgary International Airport) or 5 g of the various soil samples were placed in 100 mL of medium (medium #1), and placed on a rotary shaker (200 rpm at 30°C). After one week, bacteria were isolated from the enrichment cultures by plating on ethylene glycol agar plates.

The rotating biological contactor (RBC) used in this study has a retention volume of 30 L, and four chambers containing 56 discs. The RBC was inoculated with 2200 mL of a mixed culture containing 11 bacterial isolates obtained from the two sampling sites. Sufficient medium was added to the RBC to produce a volume of up to 30 L.

The effect of pH on growth and ethylene glycol degradation was determined in shake flask culture. An inoculum from the RBC was added to flasks of medium containing 3.65 g ethylene glycol per liter at five different pH values. At various time intervals bacterial growth/optical density at 630 nm was determined, and when the experiment was terminated, pH, bacterial growth, and ethylene glycol concentrations were determined.

The following bacteria were isolated from the enrichment cultures: *Enterobacter agglomerans*, *Acinetobacter* sp; *Pseudomonas cepacia*, *Pseudomonas fluorescens*, *Pseudomonas putida*, and an *Alcaligenes* sp.

The optimal pH for growth and glycol degradation was 5.0 (Table 7).

Colonization of the RBC discs by the bacteria was very slow for the first two weeks, but some growth was observed by the third week. During the third week a strong ammonia smell was noticed. Hydrolysis of the urea in the RBC would result in increased pH and ammonium ion concentrations, which would cause the evolution of ammonia. The influent medium was modified to correct this problem. The urea concentration was decreased by a factor of two and the pH was lowered to 5.5.

The degradation rate of ethylene glycol in the RBC was fairly low (Table 8), but as biomass continues to accumulate the degradation rate should increase. Predominantly one organism, a *Pseudomonas putida*, was isolated from various locations in the RBC.

Biodegradation of Organic Compounds in Mineral Processing Circuits

Various organic reagents used in mineral processing and other impurities from the ore make the process water unsuitable for reuse. These organic compounds tend to interfere with the flotation circuit. If the organic compounds could be microbially degraded, a significant portion of the process water could be recycled. The use of an RBC to biologically treat mine process waters is currently being investigated. The following organisms that could be used in an RBC-based system have been isolated from a sample of mine process water: *Pseudomonas cepacia*, *Klebsiella oxytoca*, *Enterobacter agglomerans*, *Acinetobacter lwoffii*, *Proteus mirabilis*, and *Providencia stuartii*.

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Table 1 – The chemistry of base metals tailings effluents

pH	2.3 – 3.9
Acidity	<1–33, 728 mg/L>
	Conc. in ppm
SO_4^{2-}	11 – 17,000
Fe	<1 – 9,860
Zn	<1 – 889
Mg	<1 – 456
Ca	11 – 710
Mn	<2 – 187
K	7 – 47
U	0 – 36
Si	<1 – 17
P	0 – 15
Se	0 – 12
Al	<1 – 15
Te	0 – 13
B	0 – 10
Na	3 – 56
Sb	<1 – 7
Th	<1 – 5
As	<1 – 16
Co	0 – 5
Pb	0 – 4
Hg	0 – 3
Sn	0 – 44
Ni	0 – 2
Mo	0 – 2
Sr	0 – 1
Cr	0 – 0.57
Cd	0 – 0.47
Cu	0 – 0.14

(Kalin, 1988)

Table 2 – Chemistry of uranium tailings drainage water

pH	2.3 – 3.9	
Acidity	2,850 – 6,500 mg/L	
Ra^{226}	1,200pCi	
	Conc. in ppm	
SO_4	5,000 –	7,500
Fe	1,000 –	2,000
Al	100 –	650
U_3O_8	<1 –	25
Th	<1 –	43
Ca	300 –	470
Cu	<1 –	7
Zn	<1 –	10
Ni	<1 –	3
Co	<1 –	3
Pb	<1 –	4
Mn	<1 –	40

(Moffatt, 1976)

Table 3 – Chemical composition of gold mill effluent, pH 10.5 – 11.2

Chemical	Conc. in ppm		
CN_{Total}	36	–	1,300
CNS^-	50	–	1,320
CNO^-	4	–	10
SO_4^{2-}	90	–	2,070
Zn	31	–	289
Cu	12	–	240
Ni	2	–	190
Fe	<0.1	–	165
Ca	0.3	–	930
Co	<1	–	6
As	0.7	–	1.0
Pb			0.8
$S_2O_3^-$	0	–	260
Cl^-	100	–	1,100

(McNamara, 1988)

Table 4 – The percentage of the population containing selenium granules during incubation in 0.1% selenite broth

Time of incubation (h)	*E. coli*	*S. heidelberg*
4	33	83
6	52	90
8	79	95
10	91	97
12	94	95
27	68	10

Table 5 – Comparison of the X-ray diffraction analysis of the selenium from *S. Heidelberg* with a selenium standard

Se°(ASTM)*		Se°(experimental)	
Diffraction, A	Relative intensity (%)	Diffraction, A	Relative intensity (%)
3.01	100	3.0	100.0
3.78	53	3.75	52.0
2.07	35	2.07	31.0
1.998	21	1.99	20.7
1.766	21	1.766	20.7

*American Society for Testing Materials Index Card No. 6-0362

Table 6 – Continuous column loading and elution of uranium

Cycle	Uptake (mg/g)	Elution (mg/g)	% Eluted
1	71.8	71.3	99
2	54.0	53.8	100
3	51.8	46.7	90
4	47.9	44.9	94
5	45.7	45.2	99
6	48.2	43.3	90
7	43.6	42.7	98
8	49.0	44.7	91
9	49.3	39.8	81
10	43.2	28.6	66
11	55.4	49.1	89
12	44.7	42.8	96

Table 7 – Effect of initial pH on bacterial growth and ethylene glycol degradation

Initial pH	Final pH	Bacterial growth – O.D. 630 nm Incubation time (h)				Ethylene glycol g/L*
		0	19	43	115	
5.0	7.71	0.27	0.79	1.02	2.34	0.38
6.0	5.58	0.22	0.69	0.82	0.93	0.78
7.0	4.90	0.25	0.59	0.68	0.85	1.13
8.0	8.15	0.22	0.55	0.52	0.47	1.70
9.0	8.14	0.26	0.62	0.52	0.37	1.93

*Initial ethylene glycol 3.6 g/L

Table 8 – Ethylene glycol concentrations of various locations in the RBC

Sample location	Ethylene glycol concentration (g/L)
Influent tank	5.50
1st chamber	4.70
2nd chamber	4.00
3rd chamber	3.50
4th chamber	3.20
Effluent	2.35

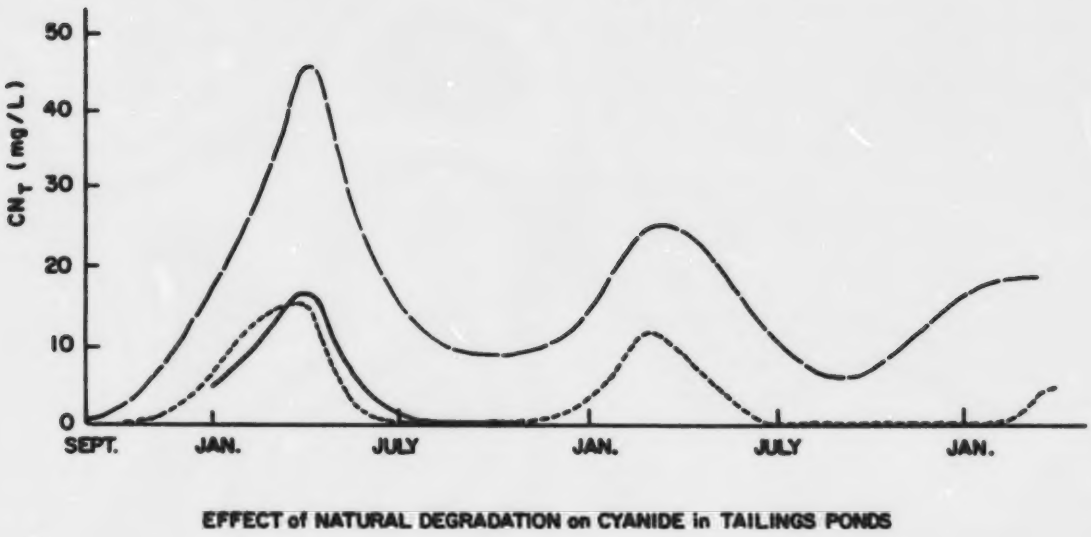

Fig. 1 – Natural degradation of cyanide at three mine sites

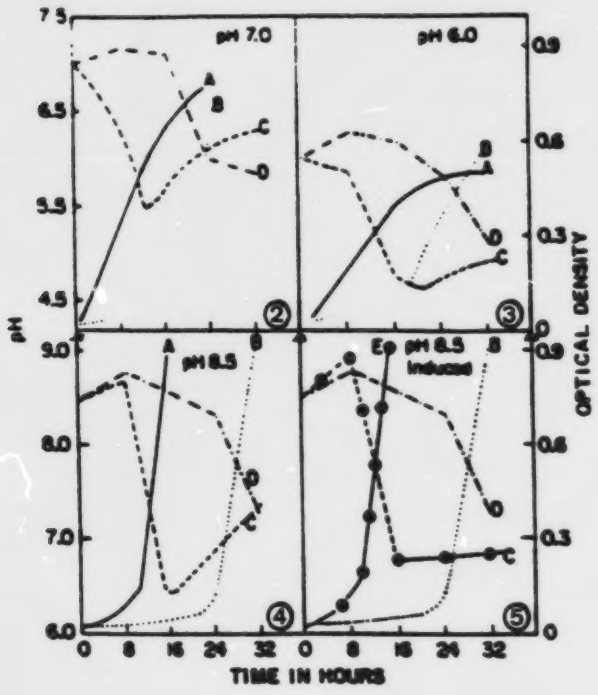

Fig. 2–5 – Effect of SeO_3^{2-} on the growth of *Salmonella heidelberg*

Fig. 2 – Initial pH 7.0. A = growth of control B = growth of selenite culture C = pH of control
D = pH of selenite culture

Fig. 3 – Initial pH 6.0. A = growth of control B = growth of selenite culture C = pH of control
D = pH of selenite culture

Fig. 4 – Initial pH 8.5. A = growth of control B = growth of selenite culture C = pH of control
D = pH of selenite culture

Fig. 5 – Initial pH 8.5 inoculated with cells previously grown on selenite
E = growth of induced cells in selenite
B = growth of non–induced cells in selenite
C = pH of induced culture
D = pH of non–induced culture

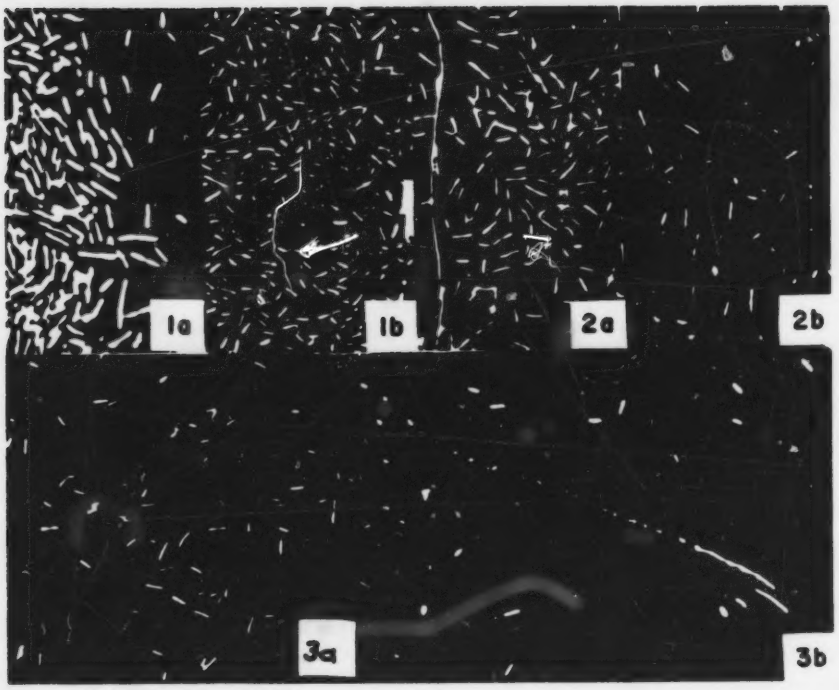

Fig. 6 – Phase-contrast photomicrograph of *Salmonella heidelberg* 1200 x. 1A – 4-h control; lb – 4-h SeO_3^{2-} culture; 2a – 8-h control; 2b – 8 – h_2-SeO_3^{2-} culture; 3a – 24-h control; 3b – 24-h SeO_3^{2-} culture

Fig. 7 – Laboratory scale rotary biological contactor

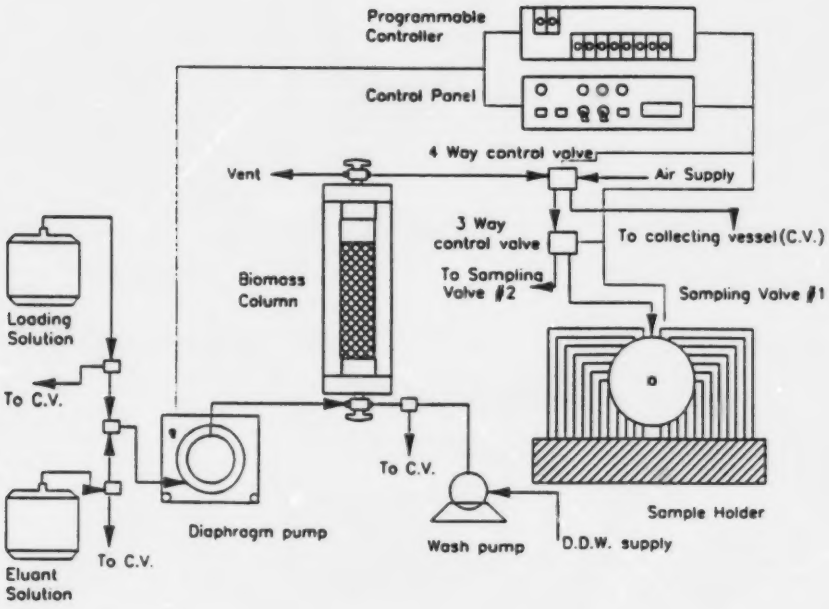

Fig. 8 – Continuous bioadsorption column system for the recovery of uranium

SESSION I : PAPER 3

BIOLOGICAL TREATMENT OF ACID MINE DRAINAGE

Anatomical and Morphological Aspects of Cattail Transplants

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ABSTRACT

Two sets of experiments were brought together under this study – the establishment of cattail populations on acidic tailings and an investigation of the ameliorating effects of organic amendments on acid mine drainage water. Both these elements are essential components in the development of a self–sustaining biological treatment process for acid mine drainage.

Cattail transplant experiments yielded varying degrees of success. The root/rhizome system was investigated from a morphological point of view to obtain evidence on the processes that lead to either death or survival of the plants. It was found that root damage during transplanting is likely to be the main factor influencing survival and, accordingly, transplanting should take place prior to root development.

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SESSION I : EXPOSÉ 3

TRAITEMENT BIOLOGIQUE DES EFFLUENTS INDUSTRIELS DANS L'ENVIRONNEMENT MINIER

Aspects anatomiques et morphologiques de la transplantation de quenouilles

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RÉSUMÉ

Les données de deux expériences différentes ont été réunies pour la présente étude : soit l'établissement de populations de quenouilles sur des résidus acides d'une part et, d'autre part, les effets mélioratifs des amendements organiques sur les effluents industriels. Ces deux éléments sont des composantes essentielles pour le développement d'un procédé de traitement biologique rentable des effluents industriels dans l'environnement minier.

Les expériences sur la transplantation de quenouilles ont été réussies à divers degrés. On a étudié la morphologie du système racine/rhizome pour obtenir des données sur les procédés qui entraînent soit la mort, soit la survie des plantes. On a trouvé que l'endommagement des racines par la transplantation est probablement le facteur principal influant sur la survie des plantes; par conséquent, la transplantation devrait avoir lieu avant le développement des racines.

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BIOLOGICAL TREATMENT OF ACID MINE DRAINAGE

INTRODUCTION

Acid generating waste sites produce seepages that require water treatment, and this is normally achieved by collecting the seepage in ponds. The water is then neutralized, either prior to leaving the pond or following treatment at a central plant to which it is pumped.

Various aspects of biological treatment systems for seepages have been addressed (Kalin and van Everdingen, 1988; Barth, 1986; Kleinmann and Heintsman, 1984; Hedin and Hammack, 1988), with particular emphasis on the use of wetlands for this purpose. A conceptual framework was recently developed for a self-sustaining treatment system, consisting of two principal components (Cairns, Kalin and Scribailo, 1988). A self-sustaining biogeochemical process for water treatment may be achieved by the establishment of vegetation which produces organic matter in the seepage flow in conjunction with microbiological sulphate reduction.

The vegetation selected for establishment in acidic seepages is *Typha* spp. The results from two series of transplant experiments in acidic conditions however, led to an impasse, as transplanted cattails produced inconsistent growth results following overwintering. However, the transplanting methods for extreme alkaline conditions were successful (Kalin, 1987). Organic amendments were made to the root/rhizome region in an attempt to ameliorate the acidity. From the survival and growth rates in the first two years, it was obvious that several factors were contributing to the failure of cattails to establish in acidic tailings, i.e., the time of transplant and/or the type of root/rhizome amendment. Cattail root/rhizome anatomy and morphology was used to determine the validity of the proposed factors, and this paper summarizes the anatomical and morphological observations made.

METHODS AND MATERIALS

DESCRIPTION OF TRANSPLANT SITES AND EXPERIMENTS

Cattails were collected from a number of locations along a spill area of an abandoned tailings site in Elliot Lake, Central Ontario. The sites at which transplants were collected represent a pH range from 1.5 to 4.5. The experiments were carried out in one or two previous growing seasons. The root/rhizome amendments from which material was collected included: no amendment (N); straw (S); and straw with lime (SL).

Transplant sites were designated as Seepage, Second Opening, First Opening and Beach, corresponding to increasing distances from the tailings mass. In addition to the collection of experimental roots and rhizomes, material was also collected from a number of additional sites, representing controls for comparison.

Root/Rhizome Collection

Cattail plants were carefully excavated from the sites using a shovel, washed to remove excess soil, and photographed to record data. The plants were then stored in plastic bags in a cooler until the following day, when they were examined or preserved for further study.

Data Collection

Observations on cattail growth were done at a number of levels. An overall assessment of the developmental status of the plants was carried out and the following parameters were measured: number of new shoots/plants; length of rhizome between new shoots and the parent plant; and height of new shoots.

After examination of all the plant material collected, selected rhizomes, roots and shoots of plants from the different sites were fixed in 70% formalin; acetic acid; 95% ethyl alcohol (FAA) in a ratio of 1:1:18. Fixed plant material was hand sectioned for anatomical evidence of deleterious effects of tailings metals on cattail growth. Thick hand sections were photographed using a Zeiss stereomicroscope SV–8 at variable magnifications.

To demonstrate the localization of metals in the rhizome tissue, we utilized a modification of the method proposed by McNary (1960). Unmordanted haematoxylin in pH 7.0 phosphate buffer binds to metals, producing a dark red colouration after several minutes of staining. Staining was particularly useful in enhancing observations on the localization of metals in thick sections of rhizomes.

Finally, some plants fixed from stage 3 were sectioned for use in scanning electron microscopy (SEM). To prepare tissue for SEM, plant material preserved in FAA was first washed in 70% ethanol before being transferred through a graded ethanol series to absolute 100% ethanol. Tissue was then critical point dried in an Omar SPC–1500 critical point dryer, mounted on metal stubs and coated for five minutes with gold palladium in a Techron Hummer V sputter coater. Material was observed at 10 kv using an Hitachi 570 SEM.

Cattail Anatomy and Morphology

Before proceeding with a discussion of the results from this study, some introductory remarks are presented to describe the basic features of the anatomy, morphology and development of cattail plants. Figure 1 diagrammatically illustrates many of these features.

Individual cattail plants consist of a rosette of opposite leaves (about 6 per side) which reproduce vegetatively through the production of underground stems (rhizomes). These rhizomes initially develop as small vegetative buds in the axil (at the base) of each leaf. Each rhizome, after a variable period of lateral growth beneath the soil, turns vertically and becomes an upright shoot. Rhizomes characteristically produce roots at regular intervals along their length, although the majority of roots tend to be produced at the bases of upright shoots.

Anatomically (in section), rhizomes consist of two distinct zones, an outer cortex and an inner pith. The pith is predominantly composed of tightly packed storage cells containing large quantities of starch granules. The cortex is largely composed of stellate (star–shaped cells) with large amounts of air space between cells. The pith is bordered on its periphery by an endodermis which acts as a barrier to lateral movement of substances from the cortex into the pith. The cortex is bordered on the outside by the exodermis which forms the outer "skin" of the rhizome and acts as a barrier between the external environment and the rhizome.

Vascular bundles are found scattered throughout the cortex and pith in particular. The primary function of vascular bundles is to transport water from the roots to the shoots and to transport sugars produced during photosynthesis from the leaves to the rhizome for storage (as starch in the pith).

The anatomy of roots is very similar to that of rhizomes, except that there is no pith region and this is replaced by a solid cylinder of vascular tissue (rather than many individual vascular bundles) termed a stele. Rhizomes produce roots laterally from the pericycle, which is a thin layer of tissue found just beneath the endodermis. Roots may also produce lateral roots from their pericycle.

RESULTS AND DISCUSSION

The morphological data obtained are summarized in Figure 2 for the entire root/rhizome collection. The observations are quantified as number of new shoots per plant, new shoot heights and rhizome length.

For the parameters length of rhizome and new shoot height, straw and straw/lime amendments showed similar development, both performing better than no amendment cattails. At the Second Opening, transplanted cattails with straw and straw/lime amendments grew better in all respects than cattails in the natural stand and natural colonization. They also performed as well as or better than control plants from the Source population and from Y1, both of the latter representing natural stands on tailings edges. Of particular interest is the fact that plants from the Seed Stand far outperformed any other plants in this investigation. The seed stand has colonized the acidic tailings from seed.

Root development was assessed by examining the level of new root initiation from the bases of new cattail shoots. These roots could be identified by their whitish colour and intact root tips. In contrast, older roots were invariably brown and heavily mineralized.

Amendment type had a dramatic effect on root development. Quantative data, comparing parameters of growth, indicate that transplanted cattails performed better the greater the distance from the tailings source (Fig. 3). The amendments had significant positive effects on cattail development. This is particularly true in the case of the straw/lime amendments, which had the greatest number of new shoots per plant at both locations, the First and Second Openings. The straw/lime amendment greatly enhanced both the extent of new root initiation and the growth of these new roots. In unamended sediments, few new roots were initiated, and those present were only a maximum of several centimetres in length. The straw treatment showed similar but slightly better development. With the straw/lime amendment, 20–30 new roots tended to be present on larger new shoots, and often these had grown as much as 10 cm in length. At the First Opening, development on the latter plants was comparable to or better than that of plants from the natural stand or that of plants from Y1.

The dramatic effect of straw and straw/lime amendments on the enhancement of root and rhizome growth is shown when one compares the treatment at the seepage (pH 1.5) no amendment with that at the same locations of straw/lime. A complete lack of roots and rhizome growth is contrasted with substantial root growth.

Overall growth, besides varying significantly between amendments versus controls, also showed a large decrease as one progressed from the Beach towards the Seepage. This decline was evidenced by an almost total loss of new shoot initiation and new root development. Deleterious effects of metals also became much more obvious towards the tailings source.

The observations on the microscopic level confirmed the earlier findings. In addition though, these results also provided considerable information about progressive stages in cattail death. This information is useful in indicating the best possible transplant method for ensuring future success.

Damage to roots and rhizomes from transplanting (i.e., loss of the growing tip) caused rapid uptake of metals through cut surfaces, leading to the rapid mineralization of roots.

It appears that new roots begin to accumulate metal oxides on their tips and external surfaces. These metals would then begin to encroach through the exodermis of the root, causing death of the exodermal layers and underlying cortex. As this accumulation increased and root tips began to blacken, a profusion of secondary roots would often be initiated and grow out just back from the tip.

These roots often exhibited abnormal growth with multiple branching occurring on the same root. Shortly after this, the primary roots would die, becoming heavily mineralized. Generally, roots showed earlier onset of this dying syndrome at sites closer to the seepage or on tailings. On cattail stands growing on the tailings, i.e., not in the seepage path, roots died rapidly, almost at initiation, leaving black scars on the rhizome or shoot base where they had previously been initiated. Within the different amendment types, this process always occurred slowest with the straw/lime amendment.

Root death was closely tied in with the death of rhizomes. Sectioned rhizomes showed first signs of mineral damage at points of entry of lateral roots into the cortex. An interesting fact to note is that this damage does not extend into the pith area, that remains white. Close examination of rhizomes with the naked eye indicated that the location of these internal zones of death could be seen externally as darkened spots appearing beneath the rhizome surface.

Anatomically, as further elucidated by haematoxylin staining, areas of metal uptake in the cortex would grow in size, until most of the cortex was blackened. Only in the final stages of rhizome death was this zone seen to encroach into the pith area. Therefore, rhizomes were often seen which had a completely black cortex but a pith that remained white. The method of slow rhizome death just described was more typically observed in cattails of less extreme conditions, such as those found in sites further away from the seepage or on the tailings, or in amended conditions. Staining of young shoots with haematoxylin identified young leaves as containing large amounts of metals, although the young developing leaves appeared to be healthy.

In sites closer to the seepage, or in places where direct contact occurred between rhizomes and tailings, death of rhizomes was more dramatic. At these locations, external damage to the rhizome surface was often visible. Metals, because of their corrosive nature, appeared to eat their way right through the rhizome surface, leading to rapid spread of metals through the cortex, with subsequent death of the rhizome following shortly thereafter.

CONCLUSIONS

Results from the anatomical and morphological study indicate that, for successful cattail establishment, the following aspects must be pursued:

The methods of plant introduction must include an assessment of the root/rhizome amendments at the time of transplanting. These can be very beneficial towards ensuring cattail growth and reproduction. This is particularly true with the straw/lime amendment, suggesting that this is the best amendment determined to date.

The overall findings from the study suggest that direct contact between roots and rhizomes with tailings results in rapid death of the organs involved. This suggests that difficulties will be encountered in

the promotion of further growth when lateral spread of cattail organs extends beyond the immediate area of the initial amendment.

Evidence from studies of iron plaque formation on cattail roots indicates that it is highly pH specific. At low pH, iron is predominantly in ferrous soluble form, and as pH increases, iron is precipitated out into the substrate (MacFie and Crowder, 1987). This explains, in part, the tendency for much greater root mineral damage (from plaque) occurring as one gets closer to the seepage. Furthermore, at a given site, the addition of lime to the tailings causes a rapid rise in Ph, resulting in precipitation of iron and metals. This, in turn, would allow root growth to proliferate without metal damage.

Another crucial factor evident from the investigation is that cattail transplant methods need to minimize the damage done to roots at the time of excavation, prior to subsequent transplant. Cut faces of roots lead to rapid mineralization and death of these roots. The process of this mineralization also leads to metal accumulation in rhizomes, the cortex and the shoot system. In both cases, vigour of the plants is undoubtedly reduced, making them more susceptible to death.

Nevertheless, evidence indicates that although localized death may occur in the cortex of the rhizomes via this method, death of rhizomes would occur only after a protracted period of exposure. A crucial factor here is whether metals can actually penetrate the pith area, causing loss of storage materials essential for renewed growth. This does not appear to be an immediate result of metal uptake by roots, since observations indicate that pith damage occurs only under extreme conditions, for example, as at the Seepage.

The observations discussed with respect to cattail establishment strongly suggest that it is imperative that the cattails be transplanted with minimal damage. The best possible method to ensure this would be to transplant cattails in the spring, prior to initiation of new roots.

An alternative approach to overcoming damage would be to use plants grown from seed. Such a strategy might overcome the additional problem of contact death described above, as follows: if seedlings are hardened during development by growth in diluted tailings sediments, it may be possible to acclimatize the seedlings to tailings with acidity and metal concentrations. Seedlings treated in this way may suffer less trauma on transplanting and exhibit more resistance when exposed to true tailings conditions. Evidence from the natural stands at the Second Opening, Y1 and Source indicates that there is certain variability in cattail resistance to metal damage.

ACKNOWLEDGEMENTS

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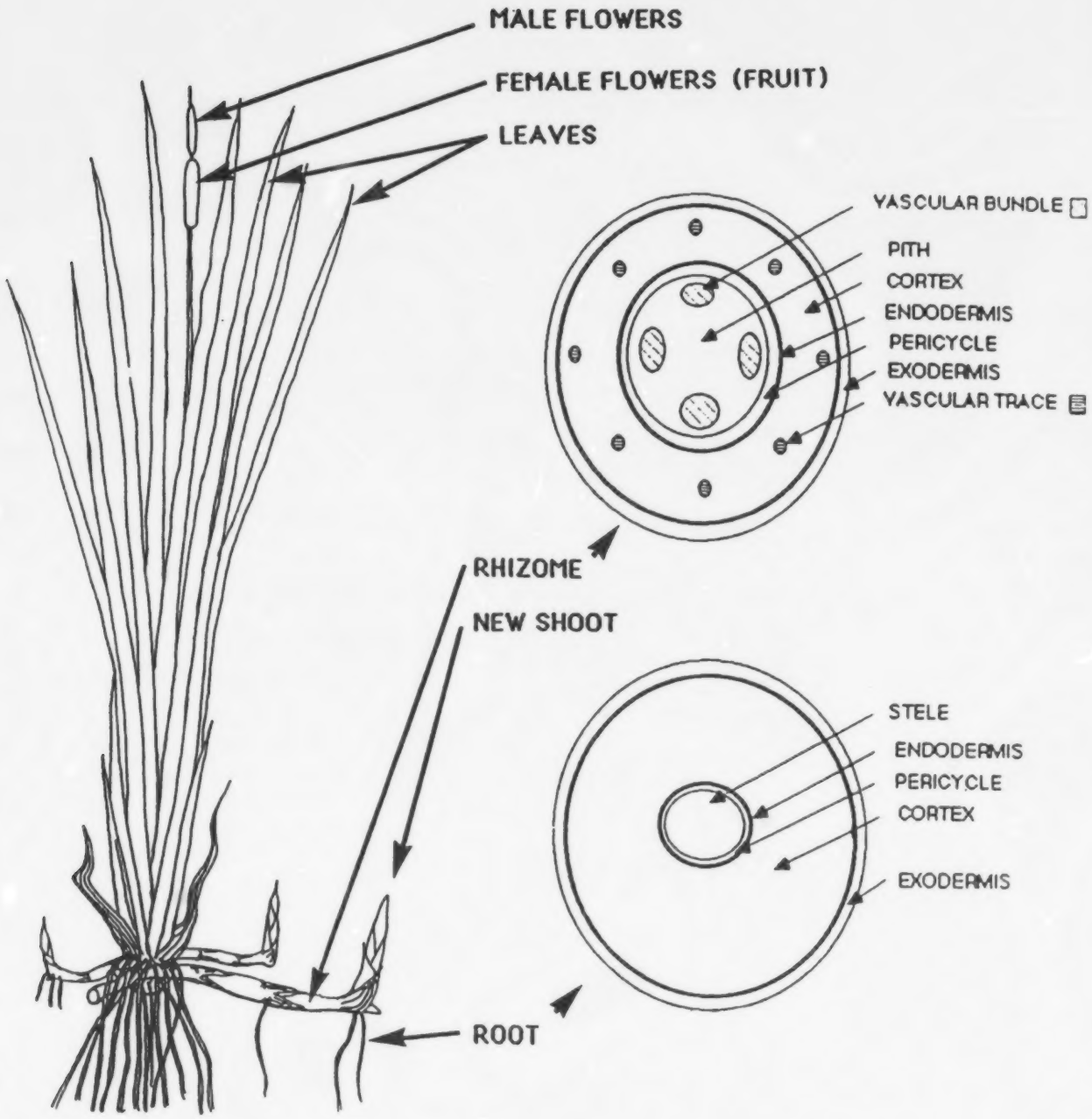
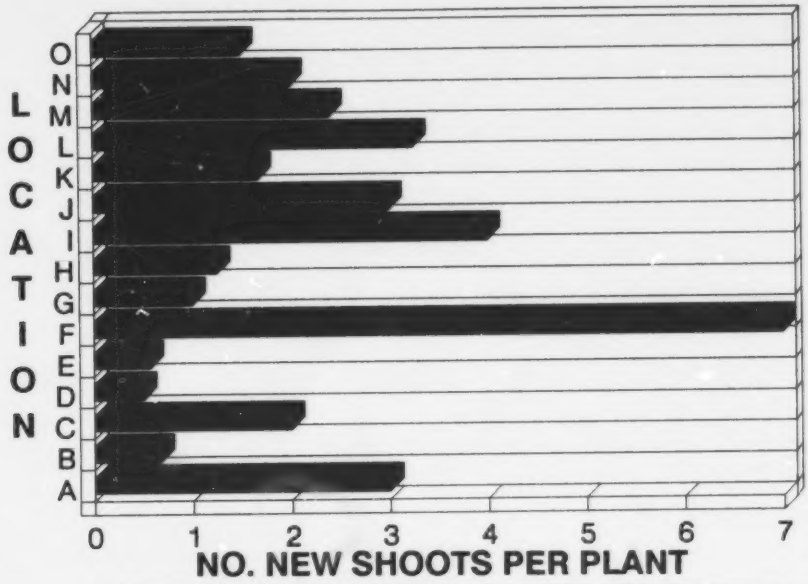
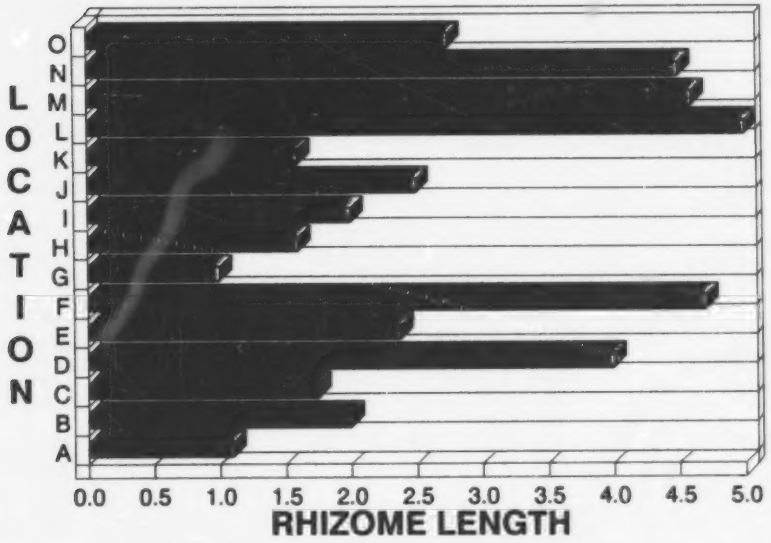


Fig. 1 – Cattail (*Typha latifolia*) plant and tissue morphology





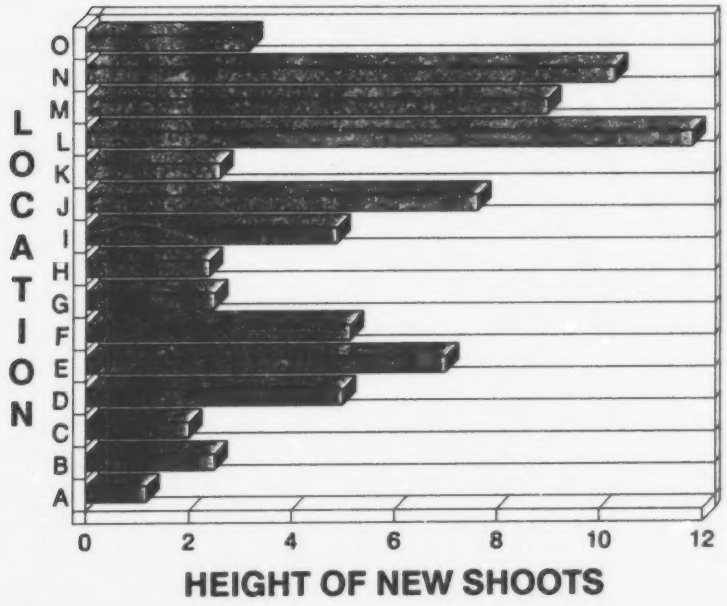


Fig. 2 – Variation in number of new shoots, rhizome length and height of new shoots with exper
tal locations

LEGEND

FIGURE 2

Growth parameters quantification of cattail root/rhizomes.

Locations on tailings:

A Natural stand on tailings spill (Y1)

B Transplanted cattails after 3 years on tailings, with lime amendment

C The source for the cattail transplants for the experiments, located on the edges of the tailings

D Transplant with straw/lime amendment in the seepage

E Transplant – no amendment in the seepage

F Seed stand on tailings

G Natural stand on tailings spill edge

H Cattails colonizing tailings from the edge of a natural stand

I Second Opening transplanted with straw/lime amendment

J Second Opening transplanted with straw

K Second Opening, transplanted with no treatment

L First Opening, transplanted with straw/lime

M First Opening, transplanted with straw

N First Opening, transplanted with no amendment

O Transplanted with no amendment onto beach

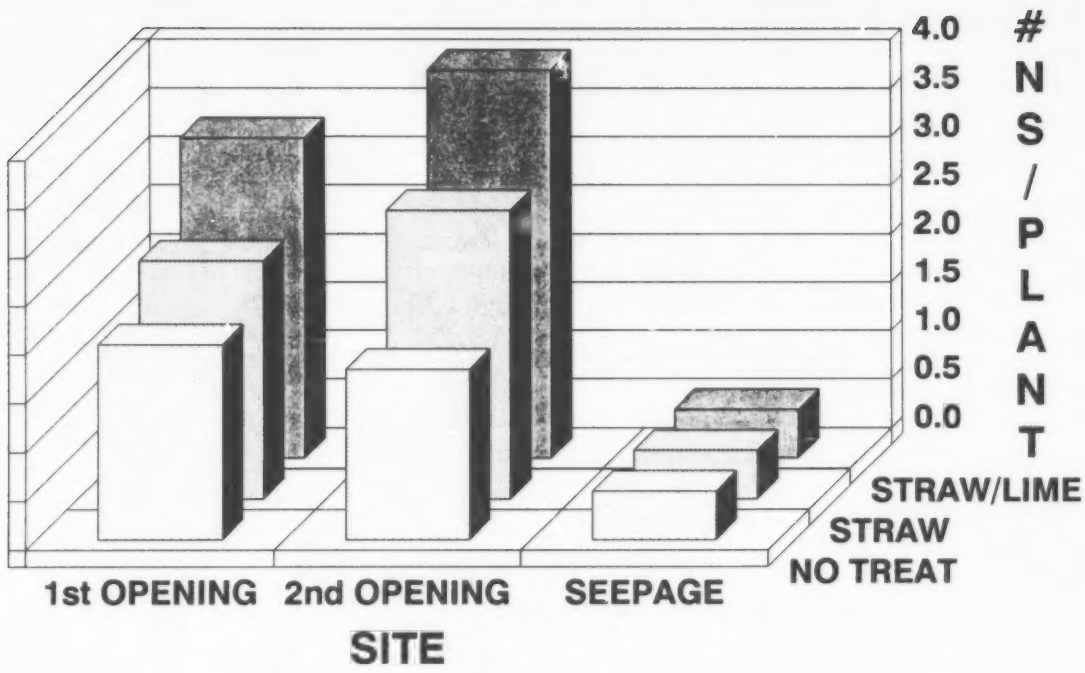


Fig. 3 – The number of new shoots initiated in transplanted cattails as a function of amendment type and transplant location. The most adverse conditions are encountered at the seepage (pH 1.5), the best conditions at the First Opening (pH 3.5).

SESSION I: PAPER 4

MICROBIAL TREATMENT FOR THE FOSSIL FUEL INDUSTRIES

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ABSTRACT

Canada's fossil fuel industries are facing strong market competition, tighter environmental regulations and a gradual shift in feedstock materials. Biotechnology and applied microbiological techniques have been commercially developed for enhanced oil recovery, flue gas desulphurization, soil reclamation, and process and waste water treatment. Researchers are also investigating microbial coal desulphurization and demetallization, enhanced solvent recovery of tar sands, biodegradation of oil, corrosion inhibition, and catalyst recovery. This study was undertaken to determine the degree of relevance of these emerging technologies to Canadian industries, the current extent of technology transfer activities in this area, and the potential for industrial applications. A review of the international scientific literature and patents was supplemented by interviews with key research and business representatives to provide the basis for this report. Enhanced oil recovery and environmental protection were found to be the main driving forces to applied research and commercial development in this area. Corporate interest in microbial applications for the fossil fuel industries appears to be increasing. There is significant opportunity for collaborative development activities.

SESSION I : EXPOSÉ 4

UTILISATION DE MICROBES DANS L'INDUSTRIE DES COMBUSTIBLES FOSSILES

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RÉSUMÉ

L'industrie canadienne des combustibles fossiles fait face à une concurrence acharnée sur le marché; de plus, elle doit se conformer à des règlements rigoureux sur la protection de l'environnement et s'adapter à un changement graduel des matières premières. Des procédés biotechniques et des techniques microbiologiques appliquées ont été développés pour rentabiliser davantage la récupération du pétrole, la désulfuration des gaz de cheminée, l'amélioration des sols et le traitement des eaux de fabrication et des eaux usées. Des chercheurs étudient aussi la désulfuration et la démétallisation microbienne du charbon, un procédé amélioré d'extraction du pétrole des sables bitumineux, la biodégradation du pétrole, l'inhibition de la corrosion et la récupération des catalyseurs. Cette étude a été entreprise pour déterminer la pertinence de ces technologies nouvelles pour l'industrie canadienne, la portée des transferts de technologie qui se font actuellement dans ce domaine et le potentiel d'application à l'industrie. Une revue des brevets et de la documentation scientifique internationale a été faite à la suite d'entrevues avec des représentants clés du monde des affaires et de la recherche; elle constitue la base du présent rapport. Les principaux motifs d'incitation à la recherche appliquée et au développement commercial dans ce domaine sont l'amélioration des techniques de récupération du pétrole et la protection de l'environnement. Les sociétés semblent porter de plus en plus d'intérêt aux applications microbiologiques dans l'industrie des combustibles fossiles. Le développement dans ce domaine offre des occasions intéressantes de participer à des projets conjoints de R-D.

MICROBIAL APPLICATIONS FOR THE FOSSIL FUEL INDUSTRIES

INTRODUCTION

McIntyre Engineering was retained by CANMET to develop an industry–driven biotechnology program to address the challenges and opportunities facing the Canadian fossil fuels industries. The program is to be operated by the Energy Research Laboratories of CANMET.

A literature and patent search was performed to determine the current state of advancement of microbial technologies for the fossil fuel industry.

Two surveys were also conducted. The first was an international survey of public and private sector researchers to determine the current level of microbiological research and development that has potential applications to the fossil fuels industries. The second was a survey of the oil, gas and coal industries to determine the current and future challenges facing Canada's fossil fuels producers.

Interviews, conducted by phone or in person, were also performed to supplement these two surveys.

In this interim report the results of our surveys and interviews are presented, and preliminary recommendations for a biotechnology program are outlined.

SURVEYS

RESULTS OF SURVEYS

In all, 68 industrial surveys and 72 research surveys were sent out by mail. Table 1 indicates the number of surveys sent to Canadian and foreign recipients, and the response rate to the surveys.

The response rate of 40% for the industry was a good result. This rate is even higher when interviews conducted just before this presentation are considered.

The response rate for the private sector researchers was low due to proprietary reasons and because many of the private sector researchers were from foreign countries where incentives to respond to the survey were few.

Table 1 – Survey response rates*

	Sent			Response	
	Canadian	Foreign	Total	Returned	Rate (%)
Industry (oil, gas, coal)	55	2	57	23	40
Industrial associations	9	2	11	3	27
Public sector researchers	14	22	36	17	47
Private sector researchers	6	30	36	6	17

*as of October 25, 1988

According to the results of the research survey, foreign laboratories are emphasizing different areas of research than are Canadian researchers. According to the frequency of citation, coal desulphurization and extraction of oil sands are the highest ranked biotechnological research activities for the foreign laboratory respondents. The highest ranked biotechnological research activities in Canada are biodegrading oil, feedstock utilization and waste water treatment. Microbial Enhanced Oil Recovery (MEOR) processes are actively being researched and developed both internationally and in Canada.

Table 2 lists fossil fuels–related biotechnological research and development activities, and compares Canadian and foreign involvement in these areas.

An interesting result shown in Table 2 exists for extraction of oil sands research. There are relatively few Canadian researchers working in this area and yet Canada has some of the world's largest oil sands deposits. In the United States this research area is receiving a surprising degree of attention in comparison with other research areas.

Table 2 – Canadian and foreign research activity rankings

Research area	Canadian	Foreign
Biodegrading oil	1	6
Feedstock utilization	2	16
Waste water treatment	3	13
MEOR: Surfactants and polymers for oil mobilization	4	3
MEOR: Selective plugging to correct reservoir conformance problems	5	9
Oil desulphurization	6	5
Cooling water	7	–
Dewatering peat	8	–
Corrosion inhibition	9	17
Soil reclamation	10	24
Microbial prospecting	11	18
Coal desulphurization	12	1
Solubilization of coal	13	12
Desulphurization of natural gas	16	22
Extraction of oil sands	17	2
Flue gas scrubbing	18	23
MEOR: Microbially secreted acids to clean–out carbonates	19	10
Extraction of oil shale	–	4
Biodegrading coal	–	7
MEOR: Repressurizing old wells	–	8
MEOR: Well–bore paraffin removal	–	11
Upgrading of coal–derived synthesis gas	–	14
Denitrification of oil	–	15
MEOR: Genetic engineering of polysaccharides	–	19
Boiler feed water	–	–
Catalyst recovery or regeneration	–	–

– : Not applicable
MEOR: Microbial enhanced oil recovery

In the industry survey, respondents were asked to list industrial processes that pose a significant bottleneck to increased profitability of their operations. These bottlenecks are listed below, in decreasing order of respondent citation.

Air quality control
Waste water quality control
Transportation of fluids
Product yield
Corrosion
Solids disposal/soil reclamation
Emulsion breaking
Product specification
Transportation of solids
Boiler feed water quality
Process water quality (including injection waters)
Occupational health and safety
Product degradation
Product losses
Catalyst poisoning
Catalyst regeneration

It should be noted that no respondents identified cooling water quality as a concern.

Based on the above survey results, application areas where biotechnology is a potentially viable solution to a problem of significant importance to industry were identified. These areas are listed below in decreasing order of perceived industrial interest.

Occupational health and safety
Air quality control
Waste water quality control
Viscosity reduction of oil for pipeline transport
Corrosion inhibition
Soil reclamation
Desulphurization (of oil, gas, coal)
Extraction of oil sands
Boiler feed water quality control
MEOR – Microbial enhanced oil recovery
Souring avoidance
Liquefaction of coals
Microbial prospecting
Petroleum upgrading
Coal and natural gas conversion

BARRIERS TO DEVELOPMENT OF BIOTECHNOLOGIES FOR THE FOSSIL FUELS INDUSTRIES

Researchers and industry representatives were asked what barriers might impede the adoption of microbial technologies within the fossil fuels industry. The various different barriers suggested are presented in the following paragraphs.

A low level of information exchange exists among researchers and organizations involved in microbial technologies. Thus new information on these technologies is very hard to obtain.

Inconsistent funding leads to delayed or incomplete research projects. According to researchers, there is a low level of funding for their respective work. Corporate executives feel research for these technologies is expensive.

There is a consensus that a lack of collaboration among industry, university and government exists and that technology transfer has not been sufficiently promoted. A low level of understanding and awareness among company executives leads to a preconceived notion that biotechnology will not play a major role in industry.

There is resistance to high risk/long-term research by many firms. Industry is hesitant to replace old technologies with new ones, even if they are proven.

Many respondents cited a lack of access to test sites as a significant barrier to development. One respondent stated that a centralized industry contact for independent researchers was required. Others cited the need for a "first player," and the technical challenges yet to be addressed fully.

PREDICTIONS FOR THE FUTURE

Biotechnology may be a major factor in solving environmental problems. Industry respondents indicated that more cost-effective methods will be required in order for industry to adhere to stricter environmental legislation.

New technologies will be required to address the shifting product requirements, and the decreasing quality and availability of feedstocks. Increased research efforts are a prerequisite to survival for the industry.

Researchers have needed that many research areas will lead to new applications for the fossil fuels industry. Biomimetic chemistry, protein engineering, genetic engineering, identification of new organisms and bioseparation all show promise as new tools for solving current and future problems.

PREFERRED METHODS OF COLLABORATION

To get an idea of the direction a biotechnology program should take, the industry representatives were asked which methods of collaboration they preferred. The five acceptable methods indicated by respondents, in decreasing order of preference, were:

Industry consortium
Joint venture with an industrial supplier
Cost shared project with government laboratory
Sponsor academic research
Contract research and development to university laboratory

PROGRAM DEVELOPMENT CONSIDERATIONS

CURRENT ROLE AND ACTIVITIES IN BIOTECHNOLOGY

The Mineral Sciences Laboratories (MSL) of CANMET has had direct and significant involvement in biotechnology research and contract administration. MSL has been involved with BIOMINET for the mineral processing industry and has funded a limited number of fossil fuels projects.

The Energy Research Laboratories (ERL) of CANMET has had no direct involvement in biotechnology research, and only limited involvement with BIOMINET and the funding and administration of biotechnology contracts for the fossil fuels industries.

PRELIMINARY RECOMMENDATIONS FOR A NEW PROGRAM

It is recommended that the new program should consist of a base program and a cost shared program.

Base Program

In conjunction with BIOMINET, collect, develop and disseminate knowledge of mechanisms, practices and procedures for the benefit of the operating companies and the Canadian public.

The focus of the base program should be on the application areas listed below:

Occupational health and safety
Management of the environment
Transportation of fluids and solids (reservoir and pipeline)
Corrosion inhibition
Hydrocarbon upgrading
Basic fossil fuels related microbiology and biochemistry (university research)

In particular, the following activities should be funded:

1. Investigations aimed at:

   - establishing standard methods to quantify the production (amounts and rates) of gases, surfactants, emulsifiers, acids and solvents, etc. under operating conditions.

   - determining the relationship between the production of various metabolites and efficiencies for various types of processes, and developing suitable models for industrial application.

   - establishing methods for compatibility testing for the prediction of in–situ microbial activity.

   - establishing guidelines for the design, conduct and reporting of field tests (including the environmental considerations of containment and waste disposal).

   - developing empirical relationships for the scale–up of laboratory data.

   - studying the long term impact on the environment (laboratory and field scale testing).

2. International technology inflow, assessment and dissemination.

3. Represent Canadian interests at international level (e.g., Environmental Protection Agency (EPA), International Energy Agency (IEA), Organization for Economic Co–operation and Development (OECD), American Society For Testing and Materials (ASTM).

4. Represent Canadian interests at the national level (e.g., Environment Canada, Energy, Mines and Resources (EMR), National Sciences and Engineering Research Council (NSERC)).

5. Expand existing Memoranda of Understanding with other levels of government to include this field of work.

6. Assemble (or accredit) and support facilities for the laboratory screening, characterization, culture collection and testing of microbial cultures for fossil fuel applications.

7. Co–sponsor symposia (with industrial representatives) and prepare briefing documents.

Cost–shared Program

A cost–shared program would need to determine and document the viability of new processes, practices and procedures. The steps required to execute the above are:

1. Work with operators to undertake pilot testing, as required by the base program.

2. Issue requests for proposals for funding.

3. Consider unsolicited proposals.

SESSION II

BIOTECHNOLOGY FOR FOSSIL FUELS

SESSION II: PAPER 5

WHAT YOU SHOULD KNOW ABOUT GOVERNMENT ASSISTANCE TO INDUSTRY

Ravi Philar, Vice President
NMR Technologies Inc.
Ottawa, Ontario

ABSTRACT

To go from concept to commercial application, every technology faces a variety of hurdles, often referred to as risk – financial, technological or human. The financial and technological risks are often interdependent, and to assist companies to overcome them, both the federal and provincial governments have created a very large – and often confusing – array of programs and incentives. Companies often face an even larger challenge: how to identify and then benefit from the programs that best meet their needs. In parallel, those who manage these programs face an equally difficult task of matching their programs to the requirements of the industry/university recipients, without lapsing funds, while ensuring that they generate adequate benefits by providing financial support. Then there are the intellectual property considerations of the funding programs and the recipients. There are, however, ways for both sides' needs to be met and for the programs to realize sufficient benefits for Canada.

SESSION II : EXPOSÉ 5

CE QU'IL FAUT SAVOIR SUR L'AIDE GOUVERNEMENTALE À L'INDUSTRIE

Ravi Philar, Vice-président
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RÉSUMÉ

Avant de passer du concept aux applications commerciales, toute technologie doit surmonter divers obstacles qui font intervenir des éléments financiers, technologiques ou humains. Les risques financiers et technologiques sont souvent interdépendants; afin d'aider les sociétés à les surmonter, les gouvernements tant fédéral que provinciaux ont créé un ensemble important (et souvent déroutant) de programmes d'aide à la recherche. Les sociétés doivent souvent faire face à un défi encore plus grand : identifier les programmes qui répondent à leurs besoins afin d'en tirer avantage. Parallèlement, les gestionnaires de ces programmes ont la tâche, tout aussi difficile, d'adapter leurs programmes aux besoins des industries/universités; sans gaspiller les fonds, mais en s'assurant que ces programmes comportent suffisamment d'avantages et fournissent l'aide financière requise. Il y a également la question de la propriété intellectuelle qui est liée aux programmes de subventions et aux bénéficiaires. Il existe cependant des façons de satisfaire aux besoins des deux parties tout en s'assurant que les programmes sont avantageux pour le Canada.

WHAT YOU SHOULD KNOW ABOUT GOVERNMENT ASSISTANCE TO INDUSTRY

FEDERAL PROGRAM ACRONYMS

UIP – University – Industry Program
IRAP – Industrial Research Assistance Program
IERD – Industry Energy Research & Development
BDP – Bioenergy Development Program
DRECT – Demonstration of Resource and Energy Conservation Technology Program
WDP – Western Diversification Program
UPP – Unsolicited Proposal Program
MDA – Mineral Development Agreements

FEDERAL ASSISTANCE PROGRAMS FOR TECHNOLOGY

OBJECTIVES:

- to reduce and share the risk in developing technology from research to commercialization
- to generate and retain most benefits in canada

KEY PROGRAM CRITERIA

UIP – University/Industry collaboration
– Economic pay–back
IRAP Laboratory Network
– Requires collaboration, technology transfer
– Clear commercial benefit
– No contribution to capital purchases
IERD – Substantial energy savings
BDP – Subtitute biomass for non–renewable fuels and chemicals
DRECT – Recovery/recycling of wastes
– Energy savings
WPD – Diversification of western economy
UPP – Uniqueness; government department sponsorship
MDA – Consensus between CANMET and provinces

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ACCESS TO FEDERAL PROGRAMS

UIP – University
IRAP Laboratory Network – CANMET
IERD, BDP – EMR
DRECT – Environment Canada
WDP – Western Diversification Office
UPP – Supply and Services (DSS)
MDA – CANMET, DSS or Province

FUNDING AND INTELLECTUAL PROPERTY

	Industry contribution to costs	I.P. ownership
UIP	100% Incremental <50% university	Negotiate with university
IRAP	>50% total 100% equipment	Industry; negotiate with subcontractors
IERD	>50% total	Industry
BDP	>50% total	Industry
DRECT	>50% total	Industry
WPD	>60% total	Industry
UPP	<50% total	Government, i.e., CANMET
MDA	>50% total	Governments

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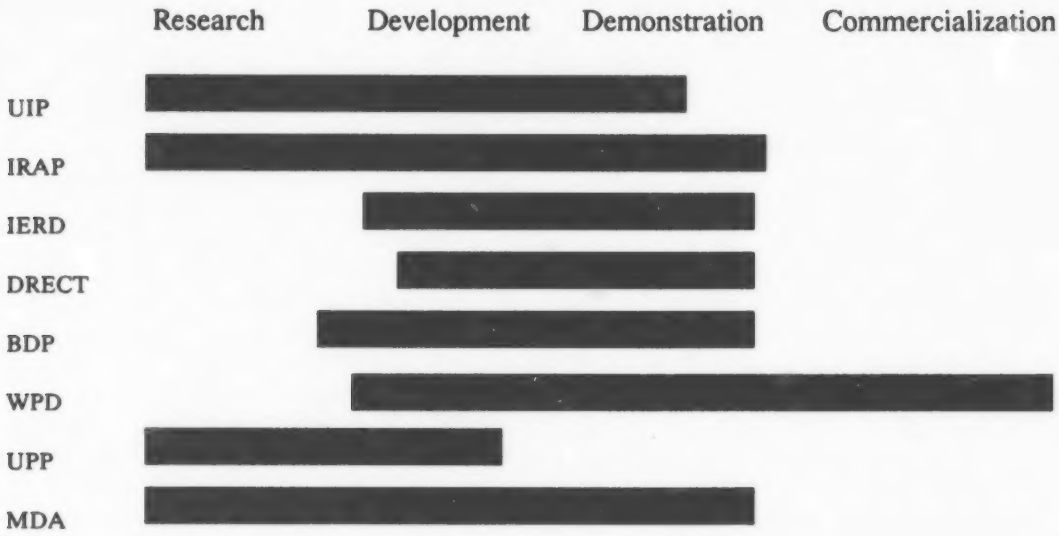
FEDERAL PROGRAMS – AREAS OF ASSISTANCE



INTELLECTUAL PROPERTY AND CONFIDENTIALITY
(Watch Out!)

Project agreements should cover:

1. Background and arising intellectual property – ownership and access
2. Publications and other disclosures
3. Post–project activities

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SESSION II: PAPER 6

BIOTECHNOLOGY AND FOSSIL FUELS

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ABSTRACT

This paper will summarize research activities in our laboratory pertaining to the effects of microbes on fossil fuel production, refining and cleanup, with emphasis on petroleum.

Microbes have been implicated in maturation of conventional oils in reservoirs with conditions suitable for growth (e.g., shallow reservoirs in contact with aquifers). This activity reduces the value of the petroleum because low molecular weight hydrocarbons are utilized. Sulphate- and iron-reducing bacteria, often associated with waterflood recovery techniques, are factors in the initiation and progression of corrosion in oil pipelines. Their incidence in oilfield liquids indicates potential corrosion/souring problems, and therefore detection and enumeration are important areas of research. Biodegradation and corrosion are usually accomplished by interactions of microbes in consortia. We are evaluating the contributions of individual strains to the overall process that results in physical and chemical changes to petroleum.

Biological treatment of petroleum (and coal) may become a feasible auxiliary process in refining. For example, biodesulphurization of high organic sulphur crude oils is under investigation, using different approaches to selectively remove thiophenic sulphur. Organic nitrogen (e.g., nitriles) in retorted shale oils is also susceptible to biological removal. Microbial deasphaltening of heavy crude oils for upgrading purposes is being studied, relying on non-specific enzymatic cleavage of the low molecular weight asphaltenes. Microbial solubilization of coal is also being evaluated.

In the environment, rehabilitation of oil spill sites occurs through the inherent degradative capabilities of natural microflora. This potential has been exploited by studying the conditions required to optimize cleanup of oil spills, such as nutrient addition and aeration. The effects of a natural oil emulsifier ("Emulsan") and synthetic oil dispersants (marine and freshwater) on oil degradation have been examined in the laboratory and in the field.

SESSION II : EXPOSÉ 6

BIOTECHNOLOGIE ET CARBURANTS FOSSILES

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RÉSUMÉ

Cette communication résume la recherche effectuée dans notre laboratoire sur les effets des microbes sur la production, le raffinage et la purification des combustibles fossiles, en particulier le pétrole.

On a observé que des microbes participaient à la maturation des huiles classiques conservées en réservoir dans des conditions propices à leur croissance (p. ex., des réservoirs peu profonds en contact avec des formations aquifères). Cette activité réduit la valeur du pétrole, car elle utilise des hydrocarbures de faible masse moléculaire. Certaines bactéries sulfato– et ferro–réductrices, souvent associées à des méthodes de récupération par injection d'eau, ont un rôle dans la formation et la progression de la corrosion des oléoducs. Leur présence dans les liquides de champs pétroliers indique la possibilité de problèmes de corrosion/acidification; leur détection et leur dénombrement sont donc des domaines importants de recherche. La biodégradation et la corrosion résultent habituellement de l'interaction de microbes vivant en association permanente. Nous sommes en train d'évaluer la contribution de chacune des souches à l'ensemble du processus qui entraîne des modifications physiques et chimiques du pétrole.

Le traitement biologique du pétrole (et du charbon) peut devenir un procédé auxiliaire de raffinage. Par exemple, la biodésulfuration du pétrole brut riche en soufre organique, fait l'objet d'une étude mettant en oeuvre différentes approches d'élimination sélective du soufre thiophénique. L'azote organique (p. ex., les nitriles) présent dans l'huile de schiste distillée, est aussi susceptible d'être éliminé de façon biologique. Dans le but d'améliorer la qualité des pétroles bruts lourds, on étudie leur désalphaltage par des microbes qui fait intervenir une scission enzymatique non spécifique des asphaltènes de faible masse moléculaire. On évalue aussi la technique de solubilisation microbienne du charbon.

Dans l'environnement, la restauration des sites de déversement de pétrole se fait par la capacité de dégradation inhérente à la microflore naturelle. Ce potentiel a été exploité par l'étude des conditions nécessaires à l'optimalisation des techniques de dépollution en cas de déversement de pétrole (p. ex. par l'addition de sels nutritifs et par l'aération). On a étudié en laboratoire et sur le terrain l'effet d'un émulsifiant naturel ("Emulsan") et d'agents dispersants synthétiques (utilisables en eau de mer et en eau douce) sur la dégradation du pétrole.

BIOTECHNOLOGY AND FOSSILS FUELS

INTRODUCTION

Microbes have been shown to play an important role in many aspects of fossil fuel (especially petroleum) technology. Although the chemistry of fossil fuels has been well studied for decades, in the last 20 years increasing attention has been directed to the biological factors influencing petroleum technology from the reservoir to the refinery. Research in our laboratory over the past 15 years has evolved as improved techniques for resolution and analysis of petroleum components have become available. The research has shifted from the observation of gross gravimetric changes in crude oil to the probing of the exquisite specificities of microbe–hydrocarbon interactions. In conjunction with an increased understanding of the individual capabilities of petroleum–degrading microbes has come an appreciation of the complex interactions of such microbes in the environment and in laboratory cultures.

Historically, the first studies were designed to determine the environmental parameters influencing biodegradation and to describe the products. Oil biodegradation is essentially an oxidative process in which *n*–alkanes and small aromatic hydrocarbons are preferentially utilized by microbes as carbon sources, producing carbon dioxide, water, cell mass and a residue. This microbial action has been shown to alter physical as well as chemical properties of petroleum. Depending on the degree of degradation, increased viscosity, decreased API°, and decreased pour point may result from depletion of the paraffins, and increased resins content may occur through accumulation of partially oxidized hydrocarbons. In general, biodegradation results in an oil of reduced quality.

Later investigations were directed towards using this information to study and exploit biodegradation. The scope of our research can be conveniently divided into four areas where microbes influence petroleum: reservoirs, recovery, refining and rehabilitation.

1. RESERVOIR MICROBIOLOGY

Reservoir microbiology is an extremely complex field. Whereas classical microbiology traditionally studies pure cultures of single organisms, often acting on a pure substrate, reservoir microbiology deals with a complex substrate consisting of hundreds of compounds in varying proportions being transformed over geological time by a shifting, mixed population of microbes with different biochemical capabilities.

Despite the tiny size of individual microbes ($\simeq 10^{-12}$ cm^3), their concerted actions are presumed to have influenced the maturation of massive volumes of petroleum in reservoirs. It is now well accepted that the Athabasca tar sands resulted from in situ biodegradation and water–washing of conventional crude oil deposits (Rubinstein et al., 1977; Crawford et al., 1978). Laboratory studies have shown that biodegradation of conventional crude oils produces residues resembling the bitumens in tar sands. As well, the bitumens are recalcitrant to further microbial alteration, as would be expected if they were the end–products of biological action.

Progressive in situ biodegradation has been inferred from related reservoirs, where alteration has resulted in crude oils of reduced quality. Three Kumak oils were obtained from increasing depths; the shallow reservoir contained oil with a gas chromatographic profile associated with biodegradation, namely *n*–alkane and aromatic depletion; the next deeper oil showed fewer chemical changes; and the deepest oil had a profile associated with unaltered petroleum (Figure 1). The deepest oil could be biodegraded by a laboratory culture to a product resembling the shallow oil (Westlake, 1983).

Since reservoir studies pose technical difficulties, the parameters influencing petroleum biodegradation in reservoirs (e.g., temperature, oxygen and nutrient requirements, pressure, pore size) have been inferred from laboratory studies and are yet to be fully understood. Although direct evidence for in situ alteration of crude oils is currently limited, it is likely that more examples will be found. Anecdotal reports of in situ souring of petroleum reservoirs (through hydrogen sulphide production) have yet to be verified. Sulphur isotope fractionation results suggest that biological action in situ does indeed occur (Semple et al., 1987).

2. PETROLEUM RECOVERY

Sulphate-reducing bacteria (SRB) have been associated with corrosion failure of oil pipelines and storage tanks and biofouling of oilfield equipment. The SRB initiate corrosion cells through localized depolarization and sustain corrosion by reducing sulphate to sulphide. Their presence in oilfields is commonly associated with secondary oil recovery procedures (e.g., waterflooding), where natural surface waters containing SRB are used as injection waters. Heavy bacterial loads and black sulphide precipitates indicate contamination of formations, production waters and oil.

Surveys of pipeline fluids from the Pembina field (Obuekwe et al., 1983) revealed numerous sulphide-generating isolates which were facultatively aerobic (i.e., able to grow with or without oxygen) in addition to the classical strictly anaerobic SRB. Under anaerobic conditions the facultative isolates reduced sulphite (but not sulphate), thiosulphate and elemental sulphur to sulphide, in a "cascade of sulphide generation" (Obuekwe et al., 1983). These "sulphide generators" are distinct from the classical "sulphide reducers", but similarly contribute to corrosion through sulphide production. In addition, they are capable of growing aerobically, thus removing oxygen from the system to reduce the local environment and protect the anaerobic SRB from oxygen.

Another group of facultative aerobes isolated from oilfield fluids has been described. This group reduces ferric iron to ferrous iron (Obuekwe et al., 1981a, K. Semple, 1987, M.Sc. thesis, University of Alberta). The ferrous iron combines with sulphide to further promote corrosion, thus acting in concert with the classical SRB and the "sulphide-generators". Some of these "iron-reducers" are also able to produce sulphide from sulphite (Obuekwe and Westlake, 1987; Westlake et al., 1986a).

None of the corrosion bacteria have been shown to degrade reduced hydrocarbons anaerobically. Rather, they appear to utilize the low molecular weight by-products of aerobic petroleum degradation (Jobson et al., 1979), such as lactate, acetate and benzoate (Pfennig et al., 1981), as carbon sources. This implies the interaction of complex consortia of aerobic oil-degrading bacteria with the facultatively aerobic "sulphide-generators" and anaerobic SRB, resulting in the reduction of iron and production of sulphides, and ultimately in the formation of anaerobic corrosion cells (Fig. 2). These consortia are closely associated with pits and "tubercles" in iron pipes, forming exopolysaccharide-mediated biofilms around the corrosion cells (Obuekwe et al., 1981b) and creating a microenvironment favorable to metal corrosion.

Since prevention and control of contamination are less costly than remediation, diagnosis and treatment of oilfield contamination in early stages may reduce economic losses due to SRB. Our laboratory is collaborating on the development of genetic probes to identify SRB in oilfield fluids and pipeline scrapings. Such probes would be incorporated into field test kits for rapid detection and identification of corrosion bacteria.

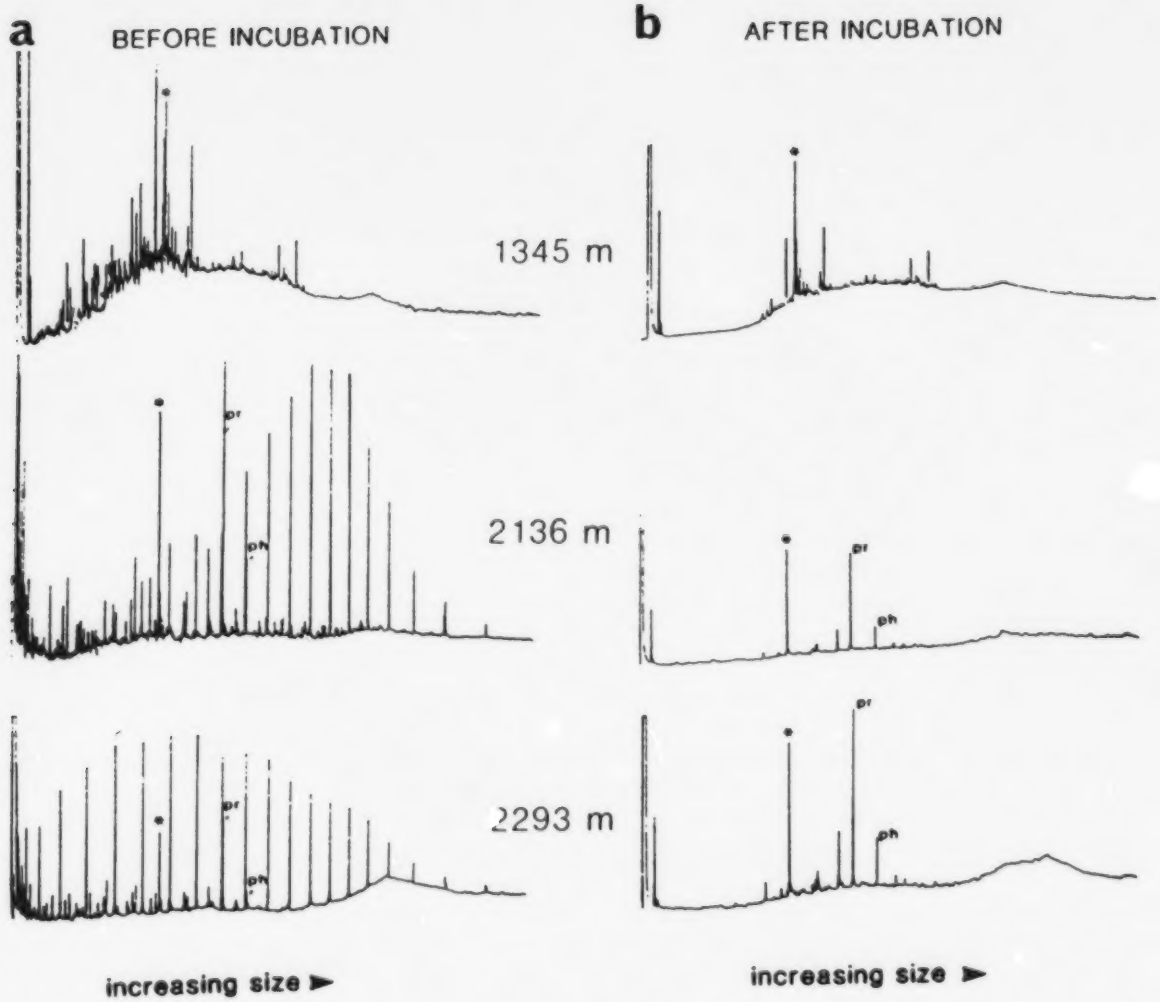

Fig. 1a – Gas chromatographic analysis of the saturated fraction of three Kumak oils from reservoirs of increasing depths. Oil from the shallow reservoir resembles biodegraded oil. (b) The oils were incubated with a mixed microbial population, resulting in similar biodegraded residues. Hexamethylbenzene, a chromatographic marker is shown by an asterisk; **pr** and **ph** denote the isoprenoids pristane and phytane, respectively. Adapted from Westlake (1983).

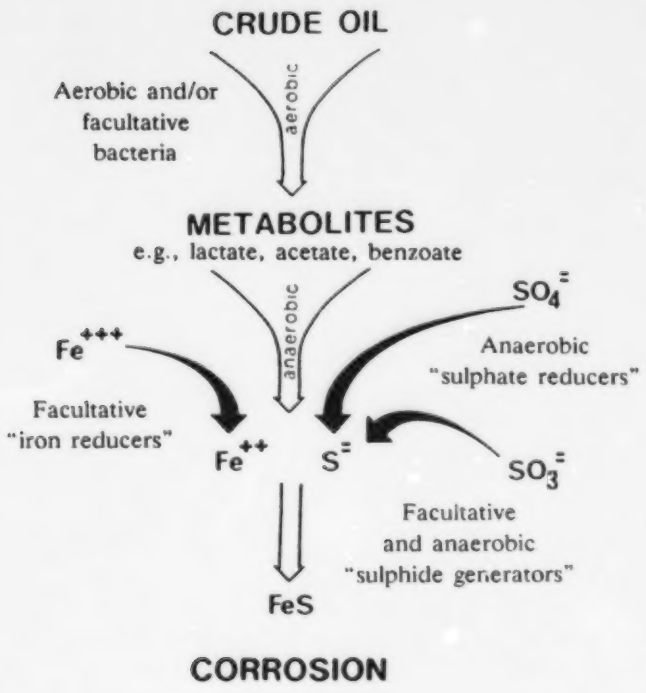

Fig. 2 – Schematic representation of the interactions of aerobic, anaerobic and facultative bacteria in the promotion and maintenance of metal corrosion in oil pipelines. Crude oil is degraded under aerobic conditions to metabolites which serve as carbon sources for corrosion bacteria under anaerobic conditions. Adapted from Westlake et al. (1986a).

3. REFINING AND PROCESSING

Several areas of interest are addressed in this section: removal of sulphur and nitrogen from oil; upgrading of heavy oils by deasphaltening; coal solubilization; and process wastewater treatment.

Sulphur is the third most common element in petroleum, and is predominantly found in organic compounds, often as thiophenic derivatives. Sulphur is presently removed from petroleum at the refinery by chemical reduction. Biological desulphurization has been proposed for a number of years as an alternative process, but has been economically unattractive for conventional oils. With decreasing supplies of low-sulphur crude oil, there has been increased interest in heavy crudes and tar sands. These feedstocks tend to have elevated sulphur levels, present especially as high molecular weight compounds in the resins and asphaltenes, and concomitantly higher chemical desulphurization costs. The feasibility of biodesulphurization is being re-examined in the light of increased understanding of biodegradation and advanced techniques for genetic engineering (Foght et al; in press; Foght et al., 1988).

Two general approaches to biodesulphurization have been studied using dibenzothiophene as a model compound. One approach relies on oxidative attack to produce polar sulphur-containing metabolites which could be water-washed from the oil (Fig. 3a). Economically, this process has the disadvantrage of removing valuable hydrocarbons along with the heteroatom in the waste stream, thus reducing the value of the oil. We have isolated a strain of *Pseudomonas* (Foght and Westlake, in press) capable

of producing water–soluble sulphur–containing metabolites from the model compound dibenzothiophene and from high sulphur crude oils (Fig. 4). This activity is not growth–dependent, and can be viewed as "biocatalysis". Genetic analysis has yielded information about the regulation of this activity. Ongoing experiments deal with immobilization of the organism onto columns, forming bioreactors through which the oil will pass for desulphurization treatment.

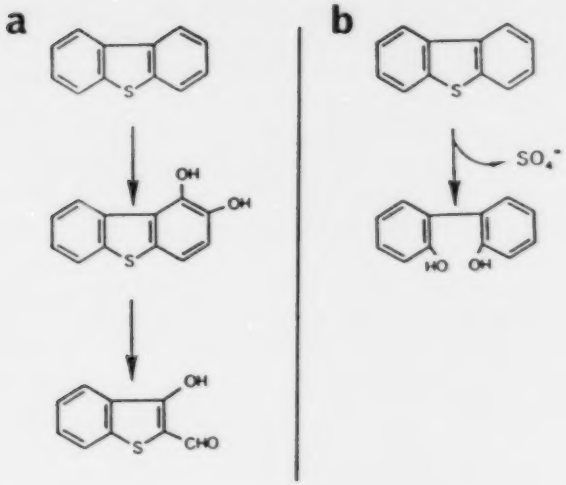

Fig. 3a – Abbreviated pathway for biodegradation of dibenzothiophene to polar product hydroxyformylbenzothiophene.

Fig. 3b – Proposed pathway for selective removal of heteroatom as sulphate. Adapted from Foght, et al.; in press.

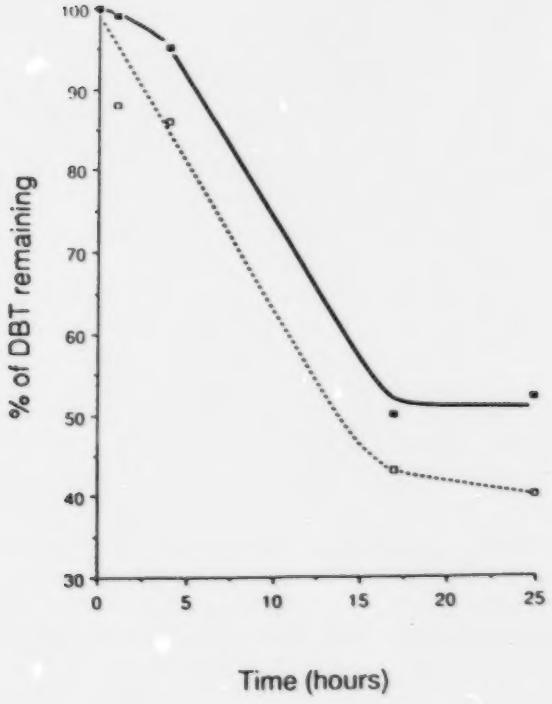

Fig. 4 – Depletion of dibenzothiophene (DBT) incubated with non–growing "biocatalyst". Residual DBT measured by gas chromatography. Broken line: crystalline DBT in suspension. Solid line: DBT dissolved in hexadecane as a model oil system. Adapted from Foght et al.; 1988.

A more recent approach to biodesulphurization uses microbes to specifically cleave the sulphur atom from dibenzothiophene producing sulphate and a partially oxidized hydrocarbon skeleton (Fig. 3b). However, this reaction has been demonstrated (Isbister et al., 1988; Kargi and Robinson, 1984) with very few organisms, and its applicability to complex organic sulphur removal is not known at present. We are currently searching for other microbial isolates capable of such action against heavy oils.

Organic nitrogen is also a potential target for biological treatment. For example, shale oils have high concentrations of nitrogen-containing compounds in addition to organic sulphur compounds. After retorting, nitriles are a major fraction of the nitrogen-containing compounds in some shale oils. The nitriles have been shown to be specifically removed from shale oil by enrichment cultures of microbes (Aislabie and Atlas, 1988). Similarly, carbazoles (Finnerty et al., 1983) and alkyl carbazoles (Fedorak and Westlake, 1984b) have been shown to be removed from conventional oils by microbial degradation.

Heavy oils and bitumens have higher asphaltene contents than conventional crude oils. It may be possible to upgrade these heavy oils biologically through oxidation and cleavage of the polynuclear aromatic ring systems to smaller molecules, thus reducing the asphaltene content (Fig. 5). An upgrading project has been initiated in our laboratory, using heavy crude oil. The asphaltenes will be fractionated into size classes and incubated with microbial cultures, and the oxidized products will be analyzed to determine the extent of size reduction (Westlake et al., 1988).

Coal can be solubilized to varying extents by microbes, facilitating transport as slurries. This process has been described in the literature (Scott et al., 1986), and relies on non-specific cleavage of

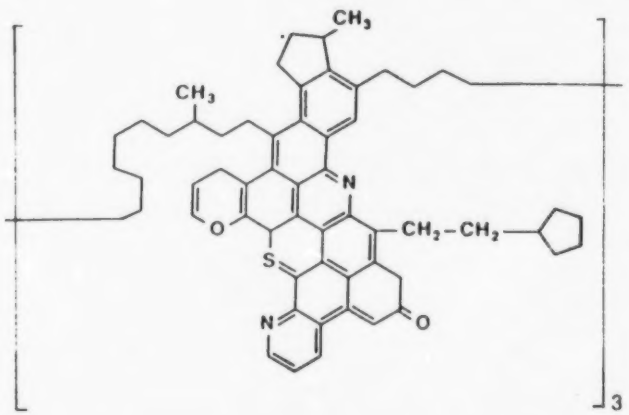

Fig. 5 – Hypothetical structure of an asphaltene. Adapted from Speight (1980)

aromatic groups from large molecular weight compounds in the coal. Typically, ligninolytic fungi have been incubated with crushed coal to produce brown-black liquids which retain almost all of their initial caloric value. Preliminary research on solubilization of low rank Alberta coals has been initiated in our laboratory.

The waste-stream waters from coal conversion processes, such as gasification and liquefaction, are noxious and require extensive treatment before discharge. Anaerobic treatment procedures for these high-strength phenolic wastewaters have been studied in detail by PMF, and are reported elsewhere in this volume.

Similarly, the wastewaters from caustic hot water extraction of Athabasca tar sands (Syncrude Canada Ltd.) are acutely toxic, and are stored in a tailings pond which has stratified into wastewater underlaid by bitumen-rich sludge. This water body must eventually be reclaimed, either through de-toxification or discharge of the wastewater and dewatering of the sludge. In order to understand the microbiology of the tailings ponds and the potential for in situ detoxification of the wastewater, a survey of the microbial content and metabolic activities in the pond was undertaken (Foght et al., 1985). Active aerobic and anaerobic microflora were detected at all depths sampled, including the sludge layer (Fig. 6a). Hydrocarbon-degradative ability was nutrient-limited in the upper layers, and very slow or non-existent in the lower layers (Fig. 6b). These results suggest that the wastewaters contain a natural microflora which could be exploited to help reclaim the upper layer of the tailings pond.

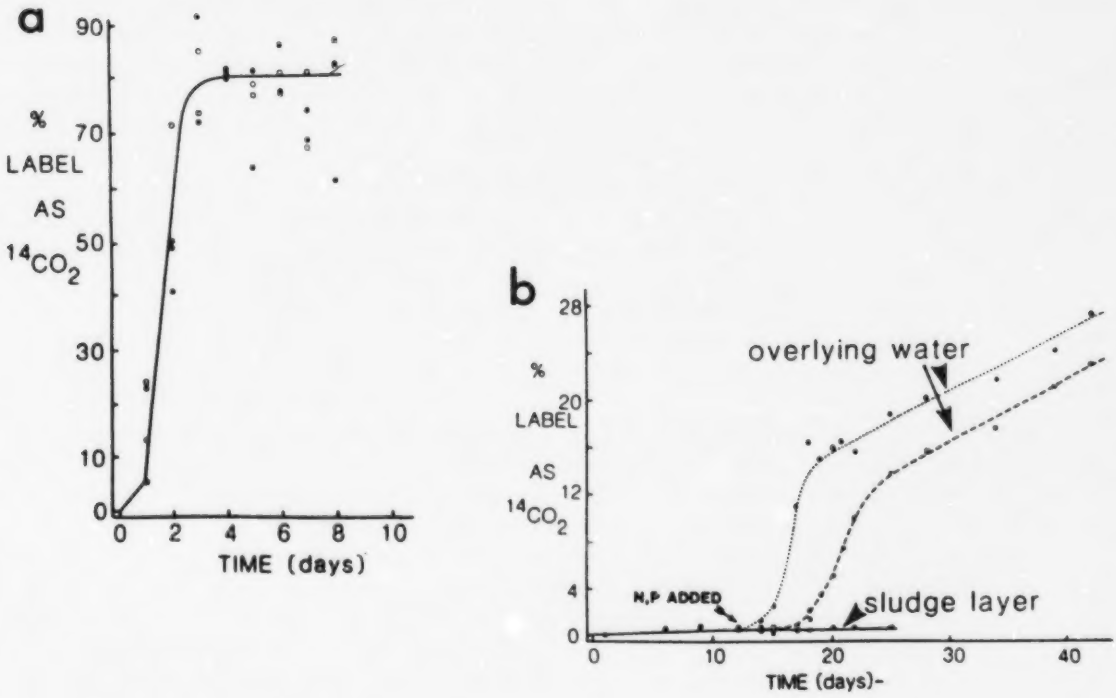

Fig. 6 – Cumulative production of $^{14}CO_2$ by samples taken from the Syncrude tailings pond at different depths and incubated with ^{14}C-substrates. (a) ^{14}C-glycolic acid. (b) [9-^{14}C]-phenanthrene. Overlying water samples are from 0.5 m and 8 m depth, and sludge samples are from 12 m and 15 m depth. Sterile nitrogen and phosphorus amendment was added to all four samples after 12 days incubation. Adapted from Foght et al. (1985).

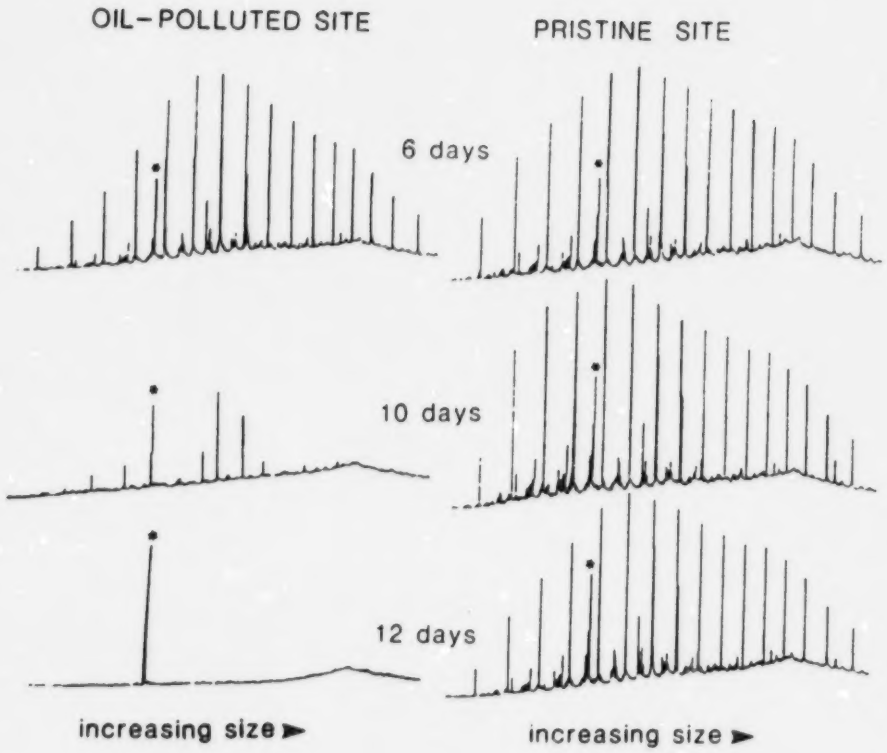

Fig. 7 – Gas chromatographic analysis of the saturated fraction of Prudhoe Bay crude oil after laboratory incubation with marine water samples. Progressive biodegradation is seen in oil incubated with the sample from a chronically oil–polluted site, whereas slower degradation is seen with the sample from a non–polluted site. The presence of hexamethylbenzene, a chromatographic marker, is shown by an asterisk. Adapted from Fedorak and Westlake (1981b).

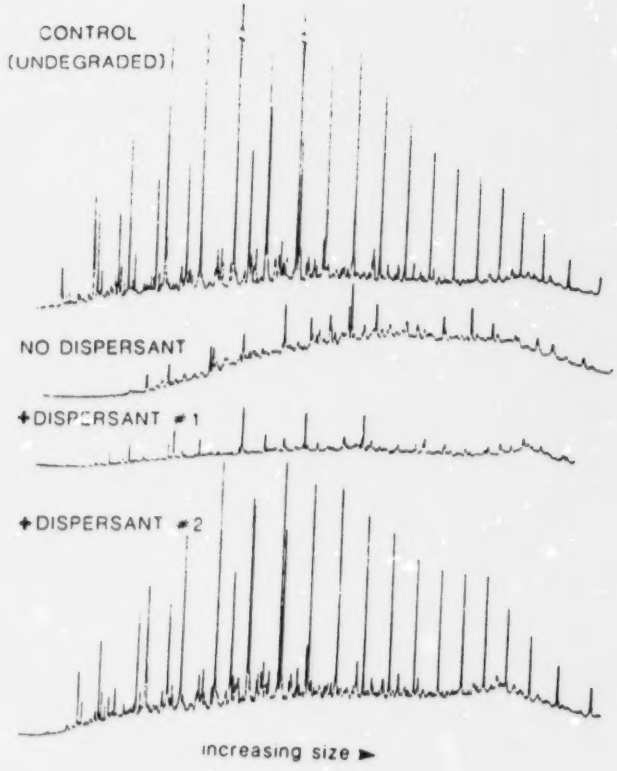

Fig. 8 – Gas chromatographic analysis of the saturated fraction of Wells crude oil after laboratory incubation with an adapted oil–degrading microbial population. Two different oil dispersants were included during incubation. Dispersant #2 inhibits oil degradation. Adapted from Foght et al. (1987).

4. REHABILITATION OF OIL SPILL SITES

Our earliest studies of microbial action on oil were related to rehabilitation of oil-soaked soil. The effects of nutrient amendments (i.e., urea-phosphate fertilizer) with or without bacterial inoculation ("seeding") were carried out in the Swan Hills, Alberta area. These field tests showed that a natural population of oil-degrading microbes already existed in the soil, which required only that nutrients be supplied in order to degrade added oil. The laboratory-grown oil-degrading bacterial inoculum did not enhance degradation of oil in the soil above the level achieved by the natural flora (Jobson et al., 1974).

The observation that natural oil-degrading microbes are ubiquitously distributed has been reinforced many times in subsequent rehabilitation studies in soil, freshwater, marine water and sediments. This was the basis for a long-term study of the oil-degradative potential of waters and sediments along the coastline in Washington state near Anacortes and Port Angeles, carried out under contract to the United States National Oceanic and Atmospheric Administration (Westlake and Cook, 1980). The activity of the microflora was correlated with the history of oil pollution in the area: microbial populations from pristine environments were less efficient at oil degradation and required some time to adapt to added oil, whereas those from chronically oil-polluted sites had populations already enriched for oil-degraders (Fig. 7). The parameters influencing oil degradation in the environment have been studied in the laboratory, and include temperature, aeration, pH, nutrients and oil quality (Lock et al., 1982; Westlake and Cook, 1980; Fedorak and Westlake, 1981a, b; Westlake et al., 1974; Jobson et al., 1972).

Chemical oil dispersants have been used to mediate marine oil spills. Unfortunately, the first generation of dispersants in some cases proved to be more toxic than the oil itself. Successive formulations were developed to be less toxic to marine life. However, at least one marine dispersant, Corexit 9527, proved to have inhibitory effects on microbial oil degradation by a mixed population (Foght and Westlake, 1982; Foght et al., 1983). Since biodegradation is a primary mode of oil removal from the environment, inhibitory effects on oil degradation are a drawback to such chemical dispersants.

The question of oil degradation inhibition arose with proposals to test freshwater dispersant formulations. Accordingly, a laboratory study was undertaken to test various dispersants for their effect on a mixed population of oil-degrading bacteria. It was found that a number of the dispersants were inhibitory to degradation at the manufacturers' recommended application rates, but some dispersants had no inhibitory or some stimulatory effects (Foght et al., 1987; Fig. 8). A field trial of dispersant Corexit 9550 was arranged for three sloughs in north central Alberta. After application of oil to two sloughs, the dispersant was sprayed onto the oil slick. Biological and chemical parameters were monitored in a multi-disciplinary study (Westlake et al., 1986b; Brown et al., in prep.). The dispersant was shown to be chemically effective and to have no adverse effects on the lower life forms in the slough, including oil-degrading bacteria.

Emulsan^{R} is a natural oil-emulsifying agent produced by a bacterium and marketed by Petroferm (USA) for various applications. One proposed use for Emulsan is the formation of water-in-oil emulsions for facilitated transport of heavy crude oils (Gutnick and Minas, 1987). Similar emulsions are currently being combusted on a commercial scale in New York state for power generation with concomitant savings in fuel consumption. Concern was raised regarding the degradability of the oil should the emulsions be accidentally spilled in the environment, and experiments were carried out to determine the effect of Emulsan on oil degradation. A mixed response was observed with pure cultures of hydrocarbon-degraders. Importantly, oil degradation by a mixed population was inhibited when the oil was emulsified (Foght, et al., 1989). This observation has stimulated further research to

see whether the inhibition is peculiar to certain oil-degrading populations or is a general phenomenon associated with Emulsan-mediated oil emulsions.

5. SPECIFIC DEGRADATION

In addition to experiments with specific applications and industrial relevance described above, we have carried out many "basic science" studies designed to understand the underlying principles of petroleum degradation. The degradative capabilities of individual bacteria (Fedorak and Westlake, 1983a; Fedorak et al., 1988; Foght and Westlake, 1988), fungi (Davies and Westlake, 1979; Fedorak et al., 1984a, Fedorak and Westlake, 1986) and mixed populations (Fedorak and Westlake, 1981a, b; 1983b; 1984a, b) have been studied with whole crude oil and individual components of crude oil. Some patterns of degradation have emerged. In general, the sequence of crude oil degradation by mixed populations follows the order: small aromatics, *n*-alkanes, larger aromatics and isoprenoids (Fedorak et al., 1983). Within the aromatic hydrocarbons, susceptibility to degradation decreases with increasing size and complexity, e.g., naphthalene > methylnaphthalenes > phenanthrene > methylphenanthrenes, etc. (Fedorak and Westlake, 1981a). This pattern is important for understanding how the quality of oil affects its degradation (Westlake et al., 1974). We have evidence (unpublished data) which suggests that bacteria are capable of degrading either aromatic or saturated hydrocarbons, but not both. This has implications in the environment where consortia of microbes are responsible for oil degradation.

The understanding of petroleum-microbe interactions gained through basic research such as this gives us a basis for predicting the fate of different crude oils based on oil composition, on the microbial population affecting the degradation, and on the physical/chemical parameters of the environment.

CONCLUDING STATEMENTS

This paper has presented an overview of some research acitvities in our laboratory related to fossil fuel biotechnology. It is by no means complete, nor does it encompass the total scope of fossil fuel research ongoing in other groups. For example, there is excellent research in the fields of: microbially enhanced oil recovery, reviewed by Jack (1988) and McInerney and Westlake (in press) and reservoir microbiology (Donaldson and Clark, 1983; Connan, 1984); biodesulphurization of pyritic (inorganic) sulphur in coal (reviewed by Monticello and Finnerty, 1985); and the use of biomarkers for petroleum exploration and petroleum diagenetic analysis (Tissot and Welte, 1984) among others.

Future areas of interest in our laboratory include microbially mediated pour point reduction in waxy crudes, microbial surfactants for bitumen recovery from tar sands, and the use of genetic engineering to enhance microbial potentials for use in fossil fuel biotechnology.

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SESSION II: PAPER 7

ANAEROBIC MICROBIOLOGICAL TREATMENT OF HIGH-STRENGTH PHENOLIC WASTEWATERS

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ABSTRACT

Anaerobic digestion has been used for over a century to treat organic sludges from municipal sewage. The improved understanding of the activities and capabilities of the microbes involved in the process have lead to its application to the treatment of industrial wastewaters. Although the food and beverage industries have the greatest experience with anaerobic treatment, its potential use for organic removal from other industrial effluents, including those from pulp and paper production, petroleum refining and coal gasification and liquefaction, has been investigated.

Our studies have focused on gaining a better understanding of the anaerobic degradation of phenolic compounds and on the application of this process to treat a wastewater from the H-coal method for coal liquefaction. This high strength effluent has a total phenolics concentration of 7600 mg/L and a chemical oxygen demand of 21 000 mg/L. It was extremely inhibitory to the methanogenic process. However, if the wastewater was diluted and supplemented with activated carbon or solvent-extracted, the major organic components, phenol, m-cresol and p-cresol could be fermented to methane. These three compounds account for 86% of the total phenolics in the waste water, and their removal by microbial activities provides a significant improvement in the effluent quality. Studies of this process have also shown that cyanide, which is commonly found in coal conversion wastewaters, is transformed to a nontoxic compound in a methanogenic consortium.

SESSION II : EXPOSÉ 7

TRAITEMENT MICROBIEN EN ANAÉROBIE D'EAUX USÉES À FORTE TENEUR EN PHÉNOLS

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RÉSUMÉ

Depuis plus d'un siècle, la digestion anaérobie est une méthode d'épuration des boues organiques des égouts municipaux. Une meilleure compréhension de l'activité et des capacités des microbes participant au procédé a entraîné son application pour le traitement des effluents industriels. Bien que ce soit l'industrie des aliments et des boissons qui ait la plus grande expérience dans la technique d'épuration anaérobie, on a aussi étudié la possibilité de l'utiliser pour l'élimination des matières organiques dans d'autres effluents industriels entre autres, ceux des usines de pâtes et papier, des raffineries de pétrole et des industries de gazéification et de liquéfaction du charbon.

Nos études visaient principalement à obtenir une meilleure compréhension des techniques de dégradation anaérobie des composés phénoliques et à les appliquer à des procédés d'épuration des eaux résiduelles de liquéfaction du charbon selon le procédé H–Coal. Il s'agit d'un effluent concentré ayant une teneur en phénols totale de 7 600 mg/L, une demande chimique en oxygène de 21 000 mg/L et qui a une action inhibitrice extrême sur le procédé méthanogène. Toutefois, lorsque les eaux résiduaires sont diluées, additionnées de charbon activé ou extraites par un solvant, les principales composantes organiques (le phénol, le m–crésol et le p–crésol) peuvent être transformées en méthane par fermentation. Ces trois composés constituent 86 % de l'ensemble des phénols des eaux résiduaires et leur élimination par des microbes entraîne une amélioration importante de la qualité de l'effluent. Des études de ce procédé indiquent également que les cyanures – qui se rencontrent souvent dans les eaux résiduaires de transformation du charbon – sont transformés en un composé non toxique par une association de microbes méthanogènes.

ANAEROBIC MICROBIOLOGICAL TREATMENT OF HIGH-STRENGTH PHENOLIC WASTEWATERS

INTRODUCTION

Organic sludges from domestic wastewaters have been treated by anaerobic digestion for over 100 years (McCarty 1982). However, industries have been reluctant to use this process because of its slow start up and its susceptibility to upset. Advances in the understanding of the microbiology and biochemistry of the process allow better selection of design parameters and loading rates to yield more stable operations. Some food industries that process meats, poultry or vegetables now successfully use anaerobic processes to treat their high-strength wastewaters.

Anaerobic processes have the following advantages over aerobic processes:

- Energy costs are lower because oxygen is not required, thereby eliminating the expense of compressing air into the bioreactor.
- Less biomass (sludge) is produced by the anaerobic process and the sludge is easier to dewater than that from an aerobic process. Therefore the ultimate disposal of anaerobic sludge is less troublesome.
- Methane, which is a metabolic endproduct of the anaerobic process, can be recovered and used as a fuel.

Recent observations have shown that anaerobic microbial populations can degrade a wide variety of phenols and chlorinated phenols (reviewed by Fedorak and Hrudey, 1988), and some simple aromatic hydrocarbons (Vogel and Grbic'-Galic', 1986; Grbic'- Galic' and Vogel, 1987). Based on these findings, research has been directed toward the application of anaerobic processes to treat phenolic wastewaters from petroleum refining (Mackenzie, 1984; Gardner et al., 1988) coal conversion processes (Harper et al., 1983; Suidan et al., 1983a; 1983b; 1987) and pulp and paper production (Benjamin et al., 1982; Hakulinen and Salkinoja-Salonen, 1982; Lee et al., 1988).

This report will focus on studies done at the University of Alberta assessing the degradation of phenols by anaerobic microbial populations. Although we have done some work on the degradation of chlorophenols (Hrudey et al., 1987a; 1987b), the anaerobic microbiological treatment of a wastewater from the H-coal method of coal liquefaction will be the major topic.

MICROBIOLOGICAL ASPECTS OF THE ANAEROBIC PROCESS

In the anaerobic process, complex organic materials are degraded by microorganisms in the absence of O_2. The major endproducts are methane and carbon dioxide. This process is the result of interactions between bacteria in a mixed population. The first group of bacteria, the "non-methanogenic population", breaks down complex molecules to acetic acid, H_2 and CO_2. These metabolic endproducts serve as major substrates for the second group, known as the "methanogenic population", which produce methane. The anaerobic process cannot function properly unless these two groups are growing under balanced conditions.

The anaerobic degradation of phenol is an excellent example of the need for both populations. The reaction of the non-methanogenic population is given by Equation 1, whereas Equation 2 summarizes the overall reaction of the anaerobic population.

$$C_6H_5OH + 5\ H_2O \rightarrow 3\ CH_3COO^- + 3H^+ + 2H_2\ \Delta G^{10} = +6.6\ kJ \qquad \text{Eq 1}$$

$$C_6H_5OH + 4\ H_2O \rightarrow 3.5\ CH_4 + 2.5\ CO_2\ \Delta G^{10} = -166\ kJ \qquad \text{Eq 2}$$

The positive free energy change in Equation 1 indicates that this reaction is thermodynamically unfavorable. Thus, if the endproducts of the non-methanogenic fermentation are allowed to accumulate, the rate of phenol degradation will be very slow. Fedorak et al. (1986) demonstrated that the addition of acetate to a phenol-degrading methanogenic consortium slowed phenol metabolism. However, if the acetate and hydrogen are consumed by the methanogens, the free energy change of the overall reaction (Equation 2) is negative and the reaction is thermodynamically favourable. This clearly illustrates the importance of the two groups of bacteria working in concert.

LABORATORY METHODS USED TO STUDY THE ANAEROBIC DEGRADATION OF PHENOLS

The serum bottle modification (Miller and Wolin, 1974) of the Hungate technique (Hungate, 1969) is used widely for laboratory studies of the anaerobic treatability or toxicity of wastewaters and pure compounds. Small culture volumes of 10 to 100 mL are routinely used with this method. Preliminary screening was done with batch cultures in which the test substrate or wastewater was added only at the time of inoculation (Fedorak and Hrudey, 1984; 1985). The method was also adapted to do semi-continuous culture studies in which a chosen volume of culture supernatant was removed daily, and this was replaced with fresh feed solution (Fedorak and Hrudey 1986a; 1986b).

During the preparation of media and feed solutions, every precaution is taken to remove all traces of O_2 from the nutrient solutions and the headspace gas in the serum bottles. To do this, the medium is boiled to expell dissolve O_2: sparged with O_2-free 30% CO_2 in N_2 while it is being cooled; each serum bottle is flushed with this gas before the medium is added; and the bottles are sealed with O_2-impermeable butyl rubber stoppers. Just prior to inoculation, Na_2S solution is added to each bottle to remove the last traces of O_2 and to further reduce the medium which contains the redox indicator resazurin that turns from pink to colorless when the Eh of the medium is below -110 mV.

Throughout our studies, anaerobic domestic sewage sludge from Edmonton was used as the inoculum and the cultures have been incubated at 37°C. The headspace gas of the cultures was monitored for methane production and the liquid medium was monitored for reductions in phenolic concentrations. Details of the gas chromatography methods for these analyses have been outlined by Fedorak and Hrudey (1983; 1984).

CHARACTERISTICS OF A COAL LIQUEFACTION PROCESS WASTEWATER

The wastewater that has been the focus of our studies came from the H-coal pilot plant at Catlettsburg, Kentucky and will be referred to as "H-coal effluent". Table 1 summarizes some of the chemical characteristics of this wastewater and Table 2 gives the concentrations of phenol and a number of alkyl phenols present in the H-coal effluent. Although not included in Table 2, small amounts of dihydric phenols were also present in this wastewater.

Not all phenolics are susceptible to anaerobic degradation. Of the compounds in Table 2, only phenol m-cresol and p-cresol have been shown to be degraded to methane. However, these three make

up 86% of the phenolics listed in Table 2. In addition, the wastewater contained a total volatile organic acid concentration (acetic to n-valeric acids) of 525 mg/L (Fedorak and Hrudey, 1986a). These compounds are readily degradable by methanogenic consortia. Dihydric phenols (catechol, resorsinol and hydroquinone) are also susceptible to anaerobic degradation.

Phenol and other substituted phenols are excellent disinfectants. Therefore it is not surprising that high strength phenolic wastewaters, such as the H-coal effluent, are usually inhibitory to microbiological processes. These wastewaters may be inhibitory for one or more of the following four reasons.

First, the degradable phenolics may be present at inhibitory concentrations. For example, well adapted (or acclimated) phenol-degrading cultures cannot tolerate phenol concentrations greater than about 3000 mg/L (J.P. Earley, Notre Dame University, personal communication). However, during the start-up phase, concentrations above 500 to 800 mg/L phenol are inhibitory.

Table 1 - Characteristics of H-coal effluent

Parameter	Concentration (mg/L)
Chemical oxygen demand	21 100
Organic carbon	7 600
Phenolics (by 4-aminoantipyrine method)	7 600
Total Kjeldahl nitrogen	270
Total phosphorus	5
Ammonia nitrogen	6.4
Nitrate nitrogen	0.8
Nitrite nitrogen	0.2
Total cyanide	0.2

Table 2 - Concentrations of phenol and alkyl phenols in H-coal effluent

Compound	Concentration (mg/L)
Phenol	4900
o-cresol	590
m-cresol	1230
p-cresol	420
2,4/2,5-Dimethylphenol	63
3,5-Dimethylphenol	210
3,4-Dimethylphenol	44

Figure 1 illustrates the effects of various phenol concentrations in batch cultures inoculated with fresh sewage sludge. The control cultures contained no added phenol and the methane produced by these cultures came from the fermentation of the organic materials which were in the sludge inoculum. After about 15 days' incubation, the cultures which contained 500 mg/L phenol began to produce more methane than the control cultures and there was a corresponding decrease in the phenol concentra-

tions. Increasing initial concentrations of phenol (1200, 2000 and 3000 mg/L) caused greater inhibition of methane production and the methane concentrations found in these cultures were less than those in the control cultures.

Fedorak and Hrudey (1984) showed that the methane bacteria are less suceptible to inhibition by high phenol or p-cresol concentrations than are the phenolic-degraders. This is quite unusual because the methane bacteria are typically the most sensitive group of microorganisms and anaerobic processes are usually designed to protect them from toxicity or other adverse environmental factors.

Second, the substituted phenolics, which are not degradable under anaerobic conditions, are usually more inhibitory than the biodegradable phenolics. For example, at concentrations of about 500 mg/L dimethylphenols are inhibitory (Fedorak and Hrudey, 1984), and at about 200 mg/L ethylphenols are inhibitory (Wang et al., 1988).

Third, numerous organic compounds are present in relatively low concentrations and these may be extremely inhibitory. In studies with H-coal effluent (Fedorak and Hrudey, 1985), part of the inhibitory nature of this wastewater was traced to ether-extractable, presumably organic, compound(s).

Fourth, in addition to the inhibitory effects of the organic compounds in these complex wastewaters, inorganic species may be present at concentrations that are detrimental to the process. These could include ammonium, sulphide and cyanide.

METHODS OF MANAGING INHIBITION

In laboratory studies on the anaerobic treatability of coal conversion effluents, there have been three general approaches to reduce the inhibitory effects of these noxious wastewaters. These have been used individually or in combination.

Dilution is the most commonly used method to reduce the toxicity of these wastewaters (Cross et al., 1982; Fedorak and Hrudey, 1985; 1986a; Nakhla et al., 1989). However, on an industrial scale, dilution becomes impractical because of the increased bioreactor size needed to treat the larger volume of diluted wastewater. A second approach used to manage inhibition is the addition of activated carbon (Harper et al., 1983; Fedorak et al., 1985; Nakhla et al., 1989). Activated carbon has been shown to adsorb phenols and unidentified inhibitory compounds, allowing higher concentrations of waste water to be treated. Suidan and coworkers (1983a; 1983b; 1987) have worked extensively with granular activated carbon anaerobic filters. A third approach, used recently by Kindzierski et al. (1988a; 1988b), is solvent extraction as a pretreatment to reduce the concentrations of phenolics and other organic compounds before the anaerobic biological process.

RESULTS FROM STUDIES WITH H-COAL EFFLUENT

EFFECTS OF DILUTION

Figure 2 shows the methane production in batch cultures that received different dilutions of the H-coal wastewater (Fedorak and Hrudey 1985). Knowing that the total phenolics in the wastewater were 7600 mg/L (Table 1) and that a phenol concentration of approximately 700 mg/L is inhibitory to unacclimated cultures, dilutions of up to 10% (v/v) were tested. Methane concentrations in excess of those produced by the control cultures, which received no H-coal wastewater, were observed in the cultures that received 2%, 4% and 6% H-coal effluent. In these three cultures, acclimation times of

12, 16 and 43 days, respectively, were required before methane production from the phenolics in the wastewater began. This phenomenon of longer acclimation times with higher phenolic concentrations has been observed in cultures fed pure phenol or p–cresol (Fedorak and Hrudey 1984). The 8% H–coal effluent concentration was only slightly inhibitory to the methanogenic fermentation of the organics in the sewage sludge, whereas the 10% H–coal effluent concentration was extremely inhibitory (Fig. 2).

The 8% H–coal effluent was equivalent to a total phenolic concentration of only about 600 mg/L in the cultures, which led us to believe that some non–phenolic compound(s) was (were) responsible for the inhibition. Further studies showed this to be true (Fedorak and Hrudey, 1985), although the identities of these inhibitors is not currently known.

Figure 3 shows results from the gas chromatographic analyses of the batch cultures which received 4% and 6% H–coal effluent. The top chromatogram shows the relative amounts of phenol, the cresols and the dimethylphenols at the time of inoculation. The gas chromatographic method did not separate m–cresol and p–cresol; therefore the peak is labeled "m/p–cresol". The middle chromatogram shows that the degradable phenolics – phenol and m/p–cresol – were completely removed from the cultures containing 4% H–coal effluent during the 66–day incubation period. After the same incubation time, virtually all of the phenol was removed from the culture which received 6% H–coal effluent but the m/p–cresol peak was still present.

Based on peak area ratios, using the non–biodegradable compound o–cresol as an "internal standard" (Fedorak and Hrudey, 1985), we concluded that only p–cresol degradation occurred in the cultures containing 6% H–coal effluent and that the "m/p–cresol" consisted solely of m–cresol (Fig. 3). Later studies using semicontinuous cultures fed dilutions of H–coal effluent showed that m–cresol was the first of the three fermentable compounds to accumulate as the process began to fail (Fedorak and Hrudey 1986a). These observations indicated that the m–cresol–degrading microorganisms are more sensitive to environmental factors than are the phenol– and p–cresol–degrading microorganisms. This prompted further investigations on the metabolism of m–cresol and culture techniques to maintain an m–cresol–degrading methanogenic consortium (Roberts et al., 1987; 1988). Considering the implications to the design and operation of a phenolic wastewater treatment process, these findings indicate that the conditions in the bioreactor must cater to the m–cresol–degrading microorganisms because they are the most fastidious group. This requirement is analogous to the current practices of anaerobic digestion, where conditions are established to cater to the methane bacteria.

EFFECT OF ACTIVATED CARBON ADDITION

Activated carbon added to microbiological processes receiving toxic wastewaters serves two major functions. First, its adsorption capacity protects the microbial population from toxic nonbiodegradable compounds and buffers the microbes from shock loads of inhibitory biodegradable compounds. Second, the large surface area of the activated carbon particles provides excellent locations for microbial attachment to form active microcolonies or biofilms.

Other groups have used relatively large bioreactors filled with activated carbon. For example, Harper et al (1983) used a 20–L reactor and Nakhla et al. (1989) used and 11–L reactor. However, we have worked with serum bottle cultures supplemented with activated carbon (Fedorak et al., 1985; Fedorak and Hrudey, 1987; Kindzierski et al., 1988b; and unpublished data). These small–scale studies allow us to test a number of dosages in a single experiment and to maintain replicate cultures during an experiment. The data in Figure 4 summarize the methane production in 10–mL batch cultures which received 10% H–coal effluent (Fedorak et al., 1985). These results clearly show that the

addition of activated carbon allows the treatment of a wastewater concentration which was otherwise extremely inhibitory.

Figure 4 also shows that the larger the activated carbon dose the shorter the acclimation time. This results from the larger amounts of phenolics being adsorbed when larger amounts of activated carbon were present (thus lower phenolic concentrations in solution). As discussed above, the acclimation times for phenol degradation are influenced by the concentration of phenol in solution. The observations from studies with an actual wastewater are consistent with those using pure phenol.

The phenolics adsorbed to the activated carbon are available to the microorganisms and these can be fermented to methane. For example, the amount of methane in replicate batch cultures containing 2% H-coal effluent and activated carbon doses of 1000 or 2500 mg/L was the same as that found in cultures without activated carbon (Fedorak et al., 1985). This process extends the useful lifetime of the activated carbon and has been termed "bioregeneration" (Suidan et al., 1980). However, this does not extend the lifetime indefinitely and replacement of activated carbon is required to prevent breakthrough of inhibitors.

SOLVENT EXTRACTION PRETREATMENT

This method has been successfully used as a pretreatment procedure to allow the aerobic activated sludge process to handle coal conversion wastewaters (Gallagher and Mayer, 1985; Humenick and Shellenbarger, 1986). Solvent extraction allows the reduction of phenolic concentrations to treatable levels. Because phenolics are weak acids, the pH of the wastewater can be adjusted to control the amount of phenolics removed by the organic solvent. For example, when the H-coal effluent was extracted with diisopropylether (DIPE) at pH 7, only 160 mg/L phenol remained in the waste water. Whereas, when the pH was adjusted to 11.5, 4720 mg/L phenol remained in the wastewater (Kindzierski et al., 1988a). In addition, solvent extraction may remove some or all of the unidentified inhibitory organic compounds from the wastewater.

Preliminary batch culture studies showed that DIPE-extraction of pH-adjusted H-coal effluent improved the treatability of the wastewater (Kindzierski et al., 1988a). The adjusted pH values ranged from 7 to 11.5. After extraction, the wastewater samples were neutralized for addition to the methanogenic consortia. The initial total phenolics concentrations in these cultures were near 750 mg/L, which was equivalent to approximately 10% (v/v) H-coal effluent. The rates of phenol degradation and the final amounts of methane produced were virtually independent of the adjusted pH of the wastewater prior to extraction.

The encouraging results from the batch culture studies lead to further work with DIPE-extracted H-coal effluent fed to 50-mL semicontinuous cultures (Kindzierski et al., 1988b). During the 250-day experiment, cultures were initially maintained on a 16.7-day hydraulic retention time and received increasing concentrations of phenolics which were governed by the pH of the DIPE extractions. Initially wastewater samples were adjusted to pH 8.5, and the resulting phenolic concentration in the feed was near 250 mg/L. After 72 days of acclimation, the feed was changed to a 1:1 mixture of pH 8.5 and 9 extracted wastewater which had a phenolic concentration of about 550 mg/L. Twenty-five days later, the cultures were fed pH 9 extracted wastewater which contained approximately 900 mg/L phenolics.

Phenol degradation was virtually complete in the semicontinuous cultures during the initial stages of these experiments (Kindzierski et al., 1988b). However, shortly after the start of the feedings with 900 mg/L phenolics, phenol began to accumulate in the cultures. The slowing of the phenolic degra-

dation rate was not due to the elevated concentrations of degradable phenolics, because they never reached inhibitory levels in the cultures. These observations suggested that the unidentified inhibitory organic compounds in the H-coal effluent were not completely removed with the DIPE extraction.

As a remedial action, 1000 mg/L of activated carbon were added to the cultures. After a period of recovery, the cultures regained their abilities to degrade the phenolics and produce methane. The loading rate was then increased to give a hydraulic retention time of 12.5 days. Figure 5 shows the influent and effluent phenol concentrations during the final stage of this experiment. These results clearly indicate the efficiency of this process.

Although the wastewater that was fed to these semicontinuous cultures was not full strength – because the solvent extraction had removed about 80% of the phenolics – it was not diluted. Thus the combination of solvent extraction and activated carbon addition allowed the treatment of undiluted H-coal effluent.

CYANIDE AND THE ANAEROBIC PROCESS

Cyanide is commonly found in coal conversion wastewaters (Neufeld, 1984) and is a potent inhibitor of methanogenesis (Smith et al., 1985). Batch culture studies by Fedorak et al. (1986) showed that in phenolic-degrading methanogenic consortia, the methane bacteria were more sensitive to cyanide inhibition than were the phenolic-degrading bacteria. However, the cultures adapted to the cyanide and converted the phenolics to methane – the higher the cyanide concentration the longer the time for adaptation. This trend was also observed in acetate-fed methanogenic cultures (Yang et al., 1980). A process for cyanide removal by anaerobic sludge was patented by Howe (1964) and discussed by Howe (1965).

Figure 6 shows the net methane production from phenol in a series of semicontinuous cultures (maintained on a 25-day hydraulic retention time), which received different concentrations of free cyanide in their feed (Fedorak and Hrudey, 1989). The amounts of methane produced in control cultures that received no phenol were subtracted from those produced in phenol-fed cultures. With cyanide concentrations which inhibited methane production, net negative values resulted, as shown in Fig. 6.

Knowing the amount of phenol fed to the cultures, the expected amount of methane was calculated and this is shown in Fig. 6. The methane production in the cultures which received no cyanide in their feed (0 mg/L) closely followed the expected yield. The presence of free cyanide in the feed solutions delayed the onset of methane production – the higher the cyanide concentration, the longer the time before methane production began. However, once methanogenesis started, the rates of methane production were virtually the same in all of the cultures.

Near the end of the 140-day monitoring period (Fig. 6), the effluents from the semicontinuous cultures were analyzed for free cyanide (Fedorak and Hrudey, 1989). None was detected, suggesting that the cyanide had been transformed to some noninhibitory compound. To verify this hypothesis, batch cultures were established containing 200 mg/L of phenol and an initial cyanide concentration of 5 mg/L. After various times of incubation, methane production and the free cyanide concentrations were measured. These results and the methane production in a control culture that received no cyanide are shown in Fig. 7. Indeed the free cyanide concentration did decrease and the onset of methane production was delayed until there was virtually no free cyanide in the cultures. Further work with $^{14}CN^-$ showed it was transformed to a radioactive product which has not been identified (Fedorak and Hrudey, 1989). The results from this work with cyanide demonstrate that not only can

anaerobic cultures remove phenolics from wastewaters, but they may also provide cyanide detoxification.

CONCLUSIONS

Laboratory studies on the treatment of high-strength phenolic wastewaters indicate that:

- the anaerobic process is capable of removing large fractions of the total phenolics and organic carbon from coal conversion wastewaters.
- a method for the reduction of inhibition is required.
- activated carbon will play a major role in this process to provide adsorption of inhibitory compounds and attachments sites for the microbial population.
- the anaerobic process must be followed by some polishing treatment to convert its effluent to discharge standards.

With appropriate development, the anaerobic methanogenic process offers considerable promise for the cost effective treatment of complex high-strength phenolic wastewaters.

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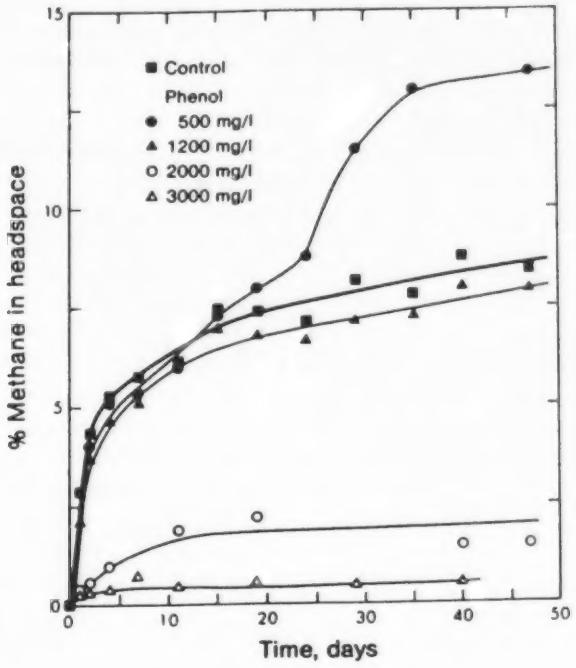

Fig. 1 – Methane production in batch cultures which received various concentrations of phenol

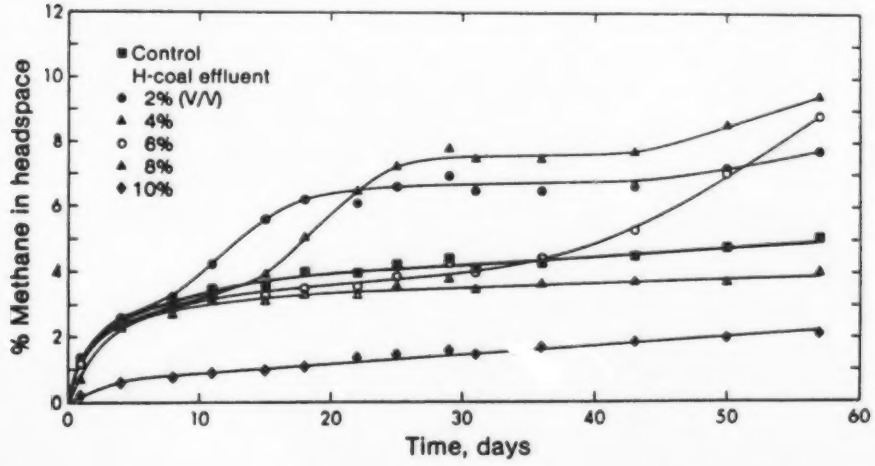

Fig. 2 – Methane production in batch cultures which received various dilutions of H–coal effluent

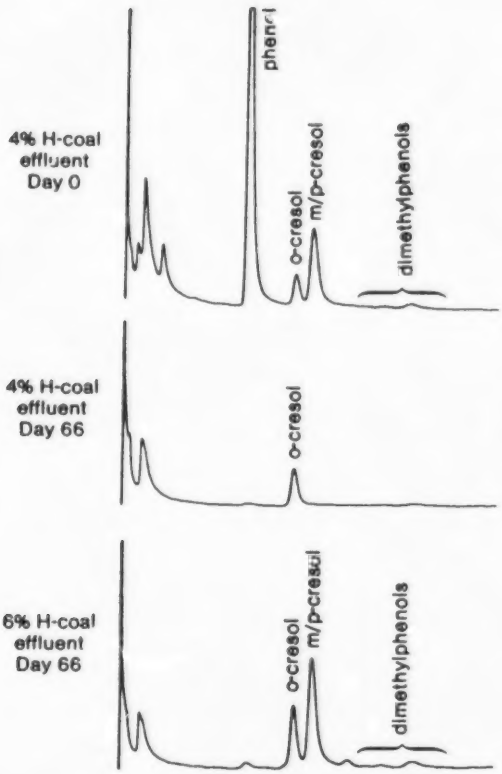

Fig. 3 – Gas chromatographic analyses of diluted H–coal effluent from batch cultures incubated for various times

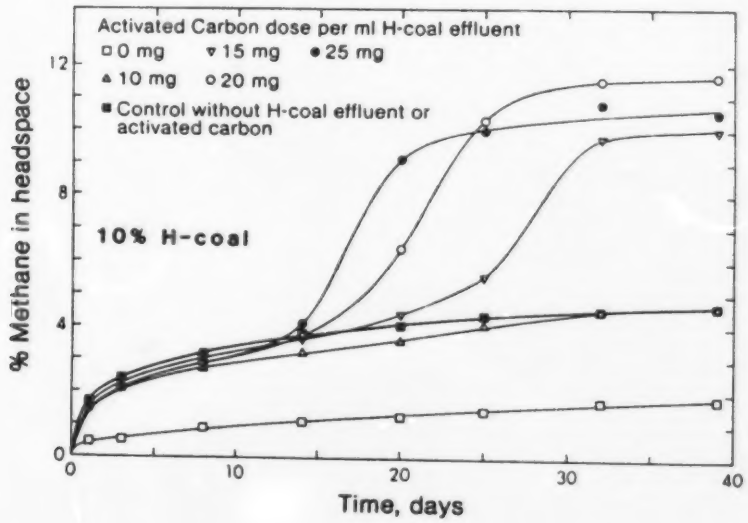

Fig. 4 – Methane production in 10–mL batch cultures containing 10% (v/v) H–coal effluent and various activated carbon dosages

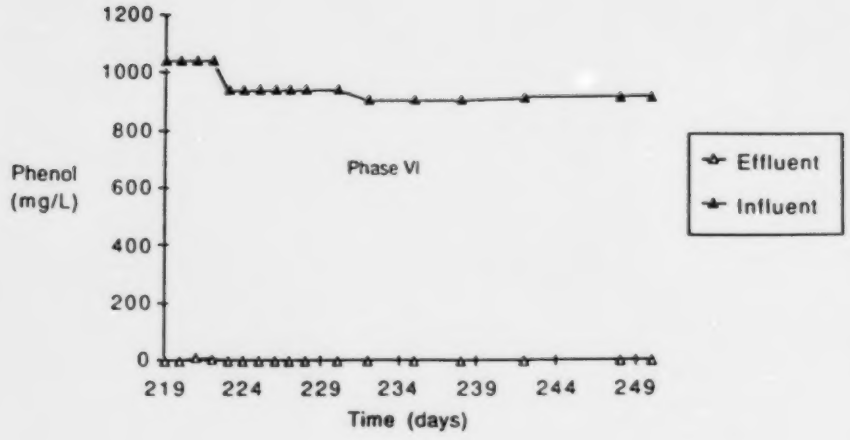

Fig. 5 – Phenol removal from undiluted, DIPE–extracted H–coal effluent in 50–mL semicontinuous cultures maintained with 12.5–day hydraulic retention time

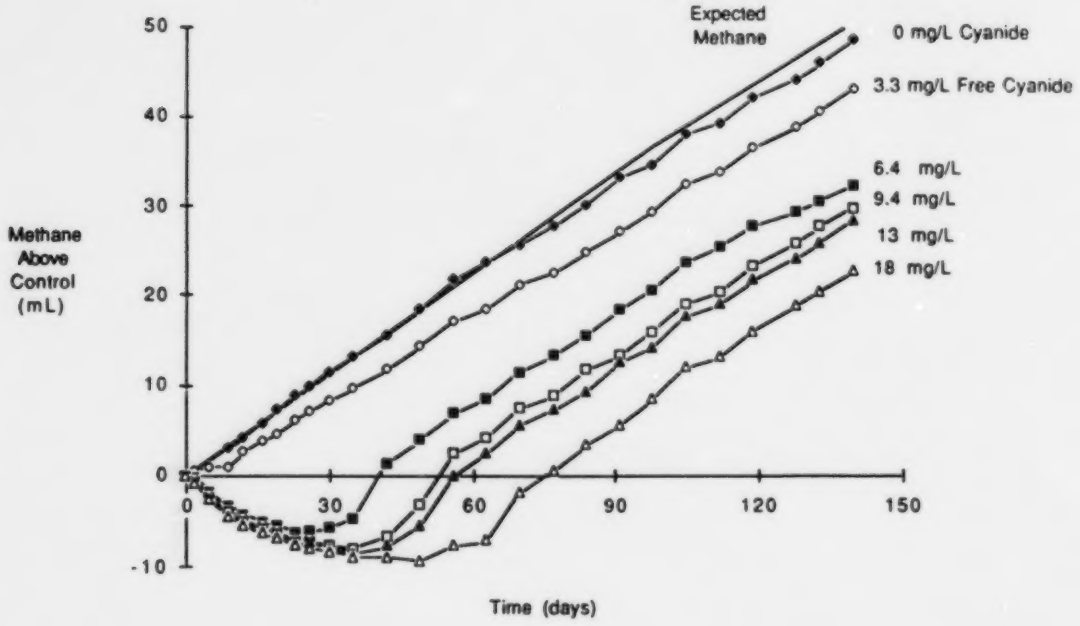

Fig. 6 – Methane production from phenol in semicontinuous cultures fed various concentrations of cyanide. The expected amount of methane that could be produced from the amounts of phenol in the feed is shown. Complexation with metal ions in the feed solution reduced the total cyanide concentrations to the free cyanide concentrations and then to those shown on the graph. The corresponding total cyanide concentrations used were (from top to bottom) 0, 10, 15, 20, 25 and 30 mg/L.

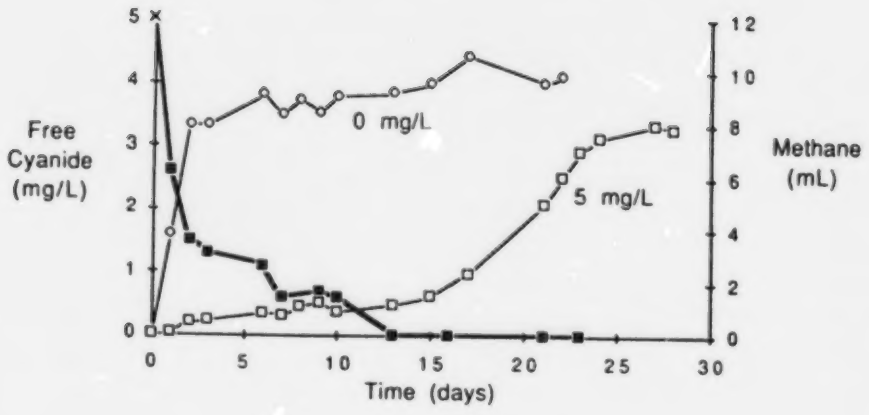

Fig. 7 – Free cyanide depletion and methane production from phenol in batch cultures that received a total cyanide concentration of 5 mg/L. The control cultures received no cyanide. "X" indicates the initial target cyanide concentration.

SESSION II: PAPER 8

LANDFARMING OF OILY WASTES
Enhancing Degradation by Specific Amendments

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ABSTRACT

Landfarming has been used for disposal of oily wastes and spilled oil in the oil industry for some time. In this process the oil is ploughed into the soil and a natural population of bacteria in the soil degrades the oil products under the influence of nitrogen and phosphorus fertilizers. Landfarming is limited by a relatively low biodegradation rate and the narrow range of oil components readily degraded. Methods are required to address these limitations. The oil absorbent peat product, Oclansorb, has been found to increase normal rates of oil degradation in both laboratory and landfarm studies. Laboratory and field studies first demonstrated that Oclansorb increased oil degradation over background levels in the presence of nutrients of nitrogen and phosphorus. Lab studies involving further additions of commercial oil–degrading preparations demonstrated increased oil degradation in one instance and no effect above Oclansorb treatment in another. Concern that commercial preparations may not persist in the environment and may have to be tailored to specific sites makes inclusion of such agents in commercial products questionable.

SESSION II : EXPOSÉ 8

INCORPORATION DE RÉSIDUS HUILEUX DANS LE SOL
Amélioration de la dégradation par des amendements spécifiques

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RÉSUMÉ

Depuis un certain temps déjà, l'incorporation dans le sol est une méthode utilisée par l'industrie pétrolière pour se débarrasser de résidus huileux et de l'huile déversée accidentellement. Ce procédé consiste à labourer le sol en y incorporant des produits huileux qui sont décomposés par une population bactérienne naturelle sous l'influence d'engrais azotés et phosphorés. L'incorporation dans le sol est limitée par un taux de biodégradation relativement faible et le petit nombre de composantes huileuses qui sont facilement dégradées. Il est nécessaire de trouver des méthodes permettant d'obvier à ces limitations. On a trouvé qu'un produit à base de mousse capable d'absorber l'huile – l'Oclansorb – augmentait la vitesse normale de dégradation de l'huile lors d'études effectuées tant en laboratoire que sur le terrain. Les deux types d'études ont d'abord montré que la dégradation naturelle du pétrole est accélérée par l'"Oclansorb" en présence de sels nutritifs azotés et phosphorés. Des études en laboratoire, faisant intervenir en outre l'addition de préparations commerciales ayant la capacité de dégrader le pétrole, ont montré une accélération du processus dans un cas et aucun effet (par rapport à l'"Oclansorb") dans un autre cas. La crainte que les préparations commerciales ne persistent pas dans l'environnement et qu'il faille les adapter à chaque emplacement remet en question l'inclusion de ces agents dans les produits commerciaux.

LANDFARMING OF OILY WASTES

INTRODUCTION

Disposal of oily wastes and sludges are of concern to the oil industry. Thousands of tons of waste oil and sludge are generated annually. A common method for disposal of this material is by landfarming it (1,2). In landfarming the waste oil is ploughed into the soil along with nutrients of nitrogen and phosphorus. Microbial action degrades the oil. The degradation rates will be dependent on soil type, moisture content, oil composition, fertilization frequency, temperature and the microbial population present. Low degradation rates and the narrow range of oil components attacked limit the landfarming process. It is now well established that the saturate alkane and simple aromatic oil fractions are readily degraded, but more complicated molecules, including the polar aromatics, are more resistant to degradation (3,4).

Methods are required to increase degradation rates. We have been looking at means of achieving this end using the oil-absorbent heat-treated peat product, Oclansorb. This oleophilic product is produced by High Point Industries. It is marketed as an absorbent for spilled oil. We were originally interested in whether this product, in addition to absorbing spilled oil, would confer any advantage in stimulating oil degradation. We were also intrigued to see how it would perform in contrast to commercial preparations of oil degrading bacteria currently available and how some of these results would compare to field trials.

METHODS

Laboratory studies were performed based on an approximation of an oil spill on a beach which had never been exposed to oil. Sand and seawater were obtained from Long Beach on Vancouver Island. To set up the laboratory study two kilograms of sand were treated with Amauligak Crude oil from a well discovered a few years ago in the Beaufort Sea. Five sets of test conditions were derived.

Test Condition	Effect
1. Amauligak oil + beach sand	Normal degradation
2. Amauligak oil + beach sand + Oclansorb	Oclansorb effect
3. Amauligak oil + beach sand + nutrients	+ Oclansorb + nutrients effect
4. Amauligak oil + beach sand + Oclansorb + nutrients + commercial oil degrader A	+ Effect of oil degrader A
5. Amauligak oil + beach sand + Oclansorb + nutrients + commercial oil degrader B	+ Effect of oil degrader B

Samples were taken from each condition over a 12-week period to measure microbial growth and oil loss patterns due to oil degradation.

In the field test, four experimental plots were obtained at a newly initiated landfarm in British Columbia. The plots were prepared thusly:

	Condition	Effect
Plot 1:	Oily sludge	Control
Plot 2:	Sludge + fertilizer + raw peat	Raw peat effect
Plot 3:	Sludge + fertilizer + Oclansorb	Oclansorb effect
Plot 4:	Sludge + fertilizer	Fertilizer effect

The plots, numbered 1 to 4, were 5 m x 7 m each. 0.8 m^3 of sludge was applied to each plot. In treatment with raw peat (153 kg) or Oclansorb (124 kg), the sludge was mixed into the peat in a mortar mixer until the oil was absorbed and the resulting mixture resembled a fine soil. This material was then spread on the plot and tilled into the soil. Fertilizer was applied to plots 2, 3 and 4 at 3.2 kg/plot. Each plot was tilled with a tractor rototiller.

Soil samples were taken for microbial counts and oil analysis over the operational period of the landfarm.

Oil from the landfarm samples was extracted by refluxing in hot toluene (the Dean and Stark extraction procedure) (5). Oil from the laboratory studies was extracted with dichloromethane. Recovered oil was separated into saturate, aromatic and polar aromatic fractions by column chromatography on silica gel, and each fraction was quantified. Gas chromatography was performed on the saturate and aromatic fractions and the patterns were compared for all experimental conditions.

Gas chromatography of recovered oil was performed with a Perkin Elmer Sigma 2000 gas chromatograph equipped with an SPB–5 capillary column (30 m x 0.25 mm ID). The head pressure of the column was 75 kPa and the splitter gas flow was 50 mL/min. The purge gas flow was 3 mL/min. The injector and detector temperatures were 320°C. The instrument was programmed to run at 50° for two minutes, then the temperature was increased by 3° per minute up to 300°, then held at this temperature for twenty minutes. The output of the data was on an HP3390A integrator with chart speed set at 0.4 cm/min. A 1–µL sample was used for injection.

Minimal sea water medium used for growth of oil–degrading bacteria from the beach experiments contained per litre: K_2HPO_4, 1.0 g; Na_2HPO_4, 1.5 g; $FeSO_4$, 0.1 g; NH_4NO_3, 1.0 g; phenol red dye, 0.018 g; filter sterilized sea water, 1 L. The final pH was adjusted to 7.0. For growth of total bacteria, the phenol red dye was omitted and this medium was supplemented with 0.5 g each of nutrient broth and yeast extract.

For the landfarm studies, minimal medium used for growth of oil–degrading bacteria contained per litre: K_2HPO_4, 10 g; NaH_2PO_4, 5 g; NH_4NO_3, 2 g; $MgSO_4.2H_2O$, 0.2 g; $CaCl_2.2H_2O$, 1 mg; $FeSO_4.7H_2O$, 1 mg; distilled water, 1 L. The final pH was 6.8. For growth of total bacteria this medium was supplemented with 0.4 g each of nutrient broth and yeast extract.

All media were dispensed at 4.5 mL per tube. Bacterial numbers were estimated by the most probable number technique. Replicate samples were serially diluted from 10^{-1} to 10^{-10}. After inoculation, 100 µL of Amauligak crude oil was added to the minimal medium tubes. All tubes were incubated at ambient temperature for 28 days. Turbid tubes indicating microbial growth were enumerated. These data were applied to standard probability tables published for this method to enumerate the number of bacteria per gram of original material (6).

RESULTS

LAB STUDIES

In laboratory studies, after five weeks' exposure, significant oil degradation occurred in the presence of Oclansorb alone as demonstrated by GC fingerprint patterns of the saturate fraction of the oil (Fig. 1). Nutrient and commercial oil degrader A increased degradation but the commercial oil degrader B did not show enhanced oil degradation.

Further analysis of the GC patterns showed that by 12.5 weeks, supplementation with oil degrader A caused the most degradation since almost all the linear alkanes were absent (Fig. 2). This was followed by oil degrader B, then Oclansorb + nutrients and then Oclansorb alone. Quantitation of the recovered saturate oil fraction and comparison with degradation occurring in the unaugmented condition showed that oil degrader A caused the most oil loss. Oil degrader B and Oclansorb + nutrients achieved the same level of oil degradation. This indicated no added benefit to adding this particular microbial culture (Table 1).

Table 1 – Oil loss in the saturate fraction from different conditions after 12 weeks

Condition	% Oil loss
Unaugmented sand	–
Oclansorb	34
Oclansorb + nutrients	44
Oclansorb + nutrients + culture A	58
Oclansorb + nutrients + culture B	42

The pattern of frequency of oil degrading bacteria in these test conditions did not correlate with the observed oil degradation pattern. Where oil degrader B was used, oil degraders disappeared from the culture vessel after two weeks and later returned. The original culture may have died off and may have been replaced later by indigenous organisms.

FIELD STUDIES

The study was moved to a landfarm to verify some of these observations. There was high variance in oil concentration in the samples from each plot. However, using composite samples for each plot, it was observed that Oclansorb caused doubling of the amount of oil degraded compared to addition of fertilizer alone (Table 2).

Table 2 – Oil loss from landfarm plots

Plot	Condition	% Oil loss
1	Sludge alone	0
2	Sludge + fertilizer + raw peat	0
3	Sludge + fertilizer + Oclansorb	25
4	Sludge + fertilizer	13

The number of oil–degrading bacteria increased and the increase was sustained in the plots treated with fertilizer and with fertilizer plus Oclansorb (Fig. 3). Increased oil–degrading numbers correlate with oil losses from these plots. Total bacterial numbers increased in all plots over the operational period, with the fastest and greatest increase in the presence of Oclansorb.

For the second season of operation each plot was sampled at the beginning of the summer season and in the fall. Analyses are now being performed. Preliminary data indicate that plot 3 treated with Oclansorb still has the least amount of oil. Data from fall sampling will be compared with this data to determine the extent of oil loss over the second season. It is interesting to note that the plot treated with Oclansorb also showed the largest amount of plant regrowth after the first operational season (Table 3) and the greatest variety in vegetation.

Table 3 – Plant regrowth in spring of second landfarm season

	Plot 1	Plot 2	Plot 3	Plot 4
Species	Horsetail fern Thistle	Horsetail fern Thistle Crabgrass Daisy Dandelion	Horsetail fern Thistle Crabgrass Daisy Dandelion Fireweed Clover Mustard Hemp nettle Plantain	Horsetail fern Thistle Crabgrass Daisy Dandelion Fireweed Clover
Relative abundance of vegetation	4	2	1	3

DISCUSSION

Laboratory experiments have shown that increased oil degradation occurs in the presence of Oclansorb, nutrients and one commercial oil degrader. Another commercial oil degrading preparation performed as well as Oclansorb plus nutrients alone. In this second preparation the oil degraders may have perished initially by two weeks in the experimental vessel and may have been replaced by an adapted indigenous population. At least they did not establish themselves readily. The implications are that universal application of commercial amendments may be uncertain. Information about the spill site and its compatibility with the commercial preparation may be required before appropriate augmentation can be applied. It appears that formulating reliable general treatments for oil spills containing specific hydrocarbon degraders for each spill scenario could pose a significant challenge. Considering the cost of these preparations and their questionable viability in the environment, Oclansorb plus appropriate nutrients of nitrogen and phosphorus may be the best general and effective amendment to enhance biodegradation.

Increased degradation was also observed in the presence of Oclansorb at the landfarm. Oil degradation was correlated with increased numbers of oil degraders. Such correlations are not always found.

One previous study noted that oil degraders which appeared in response to an oil spill were not degrading the oil but instead were living on the metabolic products of other organisms killed by the oil (7). The oil degraders probably dominated the resulting population, even though little oil degradation was occurring, due to their tolerance for the oil. Increased oil degradation in the plot treated with Oclansorb was also correlated with plant regrowth after one season's landfarm operation. This implies that after Oclansorb treatment at an oil spill site, revegetation may be aided by this treatment.

Oclansorb's mode of action in stimulating oil degradation is unknown. It does function differently from, and better than, raw peat. It does not retain increased moisture in the soil, since the water content of the experimental plots at the landfarm did not correlate with Oclansorb or raw peat treatment. One possibility is that Oclansorb has a different surface energy than raw peat and may thus bind more bacteria to its surface and facilitate degradation. Additionally, because of its oleophilic nature, Oclansorb may retain oil more effectively than raw peat, thus reducing the effective oil concentration in the soil and minimizing its detrimental effect on soil microorganisms. Because Oclansorb is so effective in promoting oil degradation, it may be the basis of a technology incorporating a contained degradation process, targeted to the oil industry.

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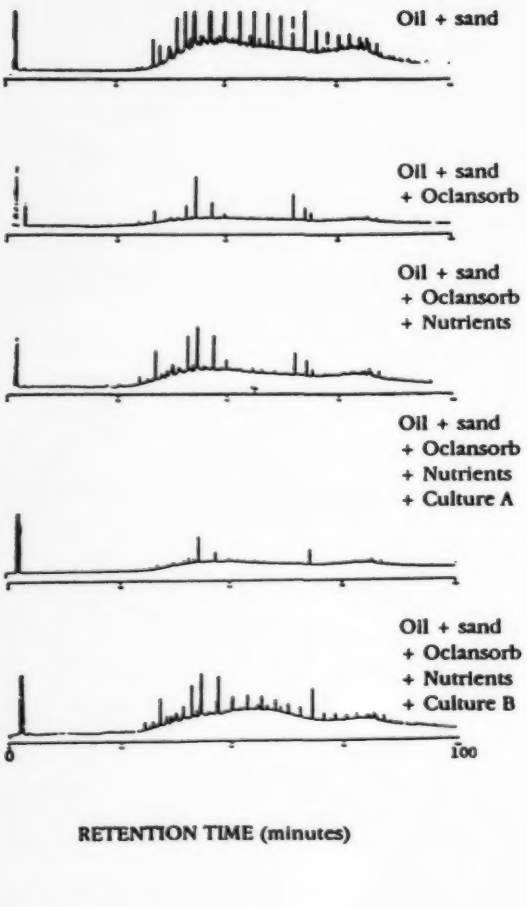

Fig. 1 – Gas chromatograms of the saturate fraction of recovered oil after five weeks of microbial exposure

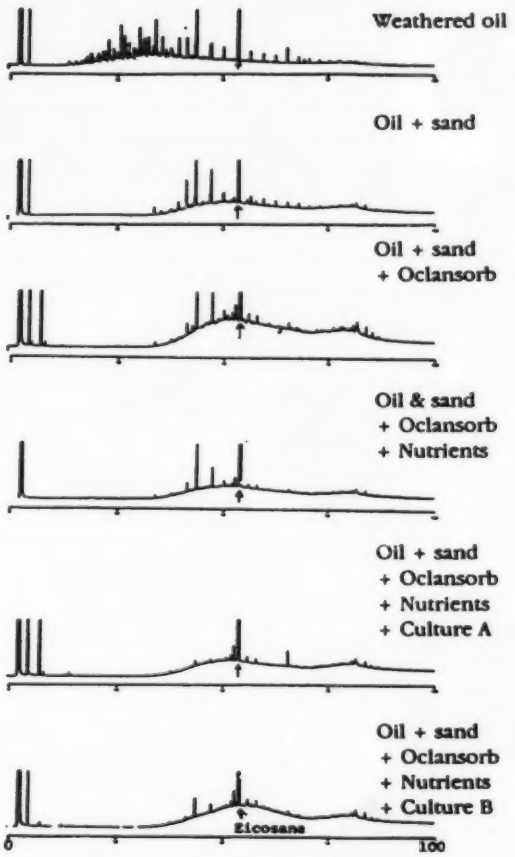

Fig. 2 – Gas chromatograms of the saturate fractions of recovered oil after 12.5 weeks microbial exposure

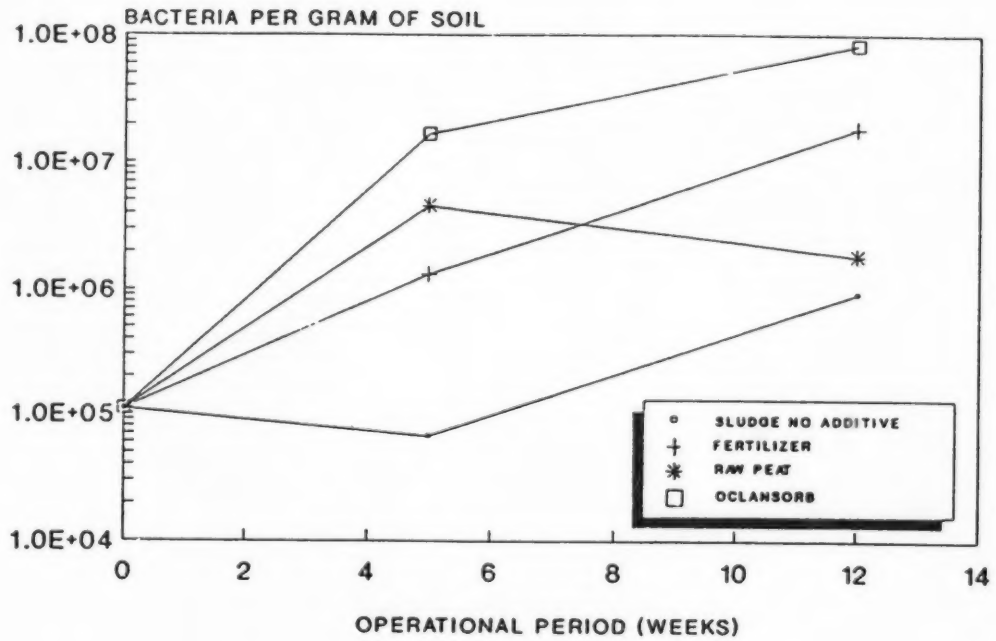

Fig. 3 – Number of oil-degrading bacteria in experimental plots over the operational period of the landfarm